Received: 15 February 2021 | Revised: 6 August 2021 | Accepted: 4 October 2021

DOI: 10.1002/wnan.1765

ADVANCED REVIEW

WIREs NANOMEDICINE AND NANOBIOTECHNOLOGY WILEY

Nucleic acid-based electrochemical biosensor: Recent advances in probe immobilization and signal amplification strategies

Krishna Thapa[1] | **Wenyan Liu**[1,2] | **Risheng Wang**[1]

[1]Department of Chemistry, Missouri University of Science and Technology, Rolla, Missouri, USA

[2]Center for Research in Energy and Environment, Missouri University of Science and Technology, Rolla, Missouri, USA

Correspondence
Risheng Wang, Department of Chemistry, Missouri University of Science and Technology, Rolla, MO 65409, USA.
Email: wangri@mst.edu

Funding information
National Science Foundation, Grant/Award Number: CCF-1814797; Ozark Biomedical Initiative Consortium Phelps Health & Missouri S&T Collaboration

Edited by: Julian Vastl, Managing Editor and Nils Walter, Co-Editor-in-Chief

Abstract

With the increasing importance of accurate and early disease diagnosis and the development of personalized medicine, DNA-based electrochemical biosensor has attracted broad scientific and clinical interests in the past decades due to its unique hybridization specificity, fast response time, and potential for miniaturization. In order to achieve high detection sensitivity, the design of DNA electrochemical biosensors depends critically on the improvement of the accessibility of target molecules and the enhancement of signal readout. Here, we summarize the recent advances in DNA probe immobilization and signal amplification strategies with a special focus on DNA nanostructure-supported DNA probe immobilization method, which provides the opportunity to rationally control the distance between probes and keep them in upright confirmation, as well as the contribution of functional nanomaterials in enhancing the signal amplification. The next challenge of biosensors will be the fabrication of point-of-care devices for clinical testing. The advancement of multidisciplinary areas, including nanofabrication, material science, and biochemistry, has exhibited profound promise in achieving such portable sensing devices.

This article is categorized under:

Diagnostic Tools > Biosensing

Diagnostic Tools > Diagnostic Nanodevices

Biology-Inspired Nanomaterials > Nucleic Acid-Based Structures

KEYWORDS

DNA nanostructure, electrochemical biosensor, nanomaterials, probe immobilization, signal amplification

1 | INTRODUCTION

The development of electrochemical biosensors has gained extensive interest due to their high detection sensitivity and selectivity, low cost, and miniaturization potential in environmental and health diagnostic applications (Chowdhury et al., 2019; Z. Li et al., 2019; Maduraiveeran et al., 2018; Nemiroski et al., 2014). Over the past several decades, a variety of nanostructured materials, such as gold nanoparticles (AuNPs), carbon nanotubes (CNTs), quantum dots (QDs), MXene, and so on have applied to and revolutionized the field of electrochemical biosensors because of their high surface-to-volume ratios and

super electrochemical properties, and thereby improved the device fabrication and greatly enhanced the sensing capabilities (Mohammed et al., 2014; J. L. Wang, Dong, et al., 2019; Y. H. Wang, He, et al., 2019; X. Wang, Niu, et al., 2019). Nucleic acids, such as DNA and microRNA, are essential materials for bodily contact. It has been demonstrated that small changes in their expression levels in biological media play a vital role in developing various diseases and cancers (Condrat et al., 2020; El Aamri et al., 2020; Swarbrick et al., 2019). Therefore, an accurate, easy-to-manufacture, and inexpensive sensing platform to monitor such biomarkers in biology and clinics is highly needed.

Nucleic acid-based electrochemical biosensors show great promise for detecting trace levels of clinically relevant analytes with high sensitivity, rapid response, and low cost. Conventionally, such biosensors are composed of three parts, namely, the analyte receptor, the signal transducer, and a data analysis system (Ronkainen et al., 2010). The receptor, generally single-stranded DNA (ssDNA) or DNA analogs (locked nucleic acid (LNA), peptide nucleic acid (PNA)) immobilized on the surface of the electrodes, is responsible for recognizing target biomarkers in solution; the transducer converts the binding interaction to a detectable signal variation, and the data analysis system interprets and displays the detected signal into a user-friendly format. In order to fabricate sensitive electrochemical biosensors, two factors are critical: one is the quality of the ssDNA probe monolayer because the density, homogeneity, and upright conformations of ssDNA probes on the electrode surface determine the accessibility of the target molecules (Pei et al., 2013; F. Yang et al., 2018), another one is the signal amplifier which amplifies the subtle interface changes into a detectable electrochemical signal (I. H. L. Cho et al., 2018). Nanomaterials have been widely employed and integrated into the fabrication of electrochemical biosensors due to their multifunctional nature for improving the sample preparation, probe immobilization, target hybridization, and signal amplification (Bezinge et al., 2020; I. H. Cho et al., 2020). Recently, the self-assembled DNA nanostructures have been extensively used as nanoplatforms to precisely organize functional nanomaterials at the nanometer scale (Jia et al., 2019; W. Liu et al., 2017; R. Wang et al., 2012). This feature with DNA natural biocompatibility further enhances DNA nanostructures' attractiveness for biosensing applications, especially the improvement of probe immobilization. Herein, we aim to review the recent advances in nucleic acid-based electrochemical biosensors, focusing on the nanomaterial enhanced probe immobilization and signal amplification strategies used for electrochemical DNA biosensors.

2 | IMMOBILIZATION OF DNA PROBES ON THE SURFACE OF ELECTRODES

The integral properties of DNA-based biosensors, including sensitivity, selectivity, and reproducibility, are highly dependent on the dispersion and orientation of the self-assembled monolayer (SAM) of DNA probes on the surface of electrodes (Teles & Fonseca, 2008). However, the construction of the DNA probe layer can face different challenges, such as unexpected surface adsorptions, disordered conformations, uneven grafting density, and the flexibility of probe molecules. Therefore, it is essential to understand immobilized DNA probes' physical structure on the electrode surface and further control the probe immobilization processes. Several studies have shown that a well-controlled inter-probe distance and an upright DNA probe conformation could efficiently increase the accessibility of target molecules and enhance the hybridization interaction between the targets and the probes, thereby leading to higher detection sensitivity (Pei et al., 2013; F. Yang et al., 2018). Different immobilization strategies have been employed to form the SAM of DNA probes.

2.1 | Direct immobilization of DNA probes

In this method, ssDNA probes are directly assembled on the electrode surface with the assistance of polymers, nanoparticles, proteins, or different functional groups. Either the electrode surface is coated with polymers/nanoparticles that can electrostatically interact with the DNA probe or one end of the probe may need to be modified with appropriate functional group like thiol (SH), aldehyde (CHO), carboxyl (COOH), amines (NH_2), and biotin/avidin, so that they can form corresponding bond on the electrode surface to immobilize ssDNA probes.

2.1.1 | Physical adsorption

Physisorption, or nonspecific adsorption, is considered the most straightforward method for immobilizing DNA probes onto the electrode surface because there is no need to modify the DNA probes. The DNA probe immobilization is

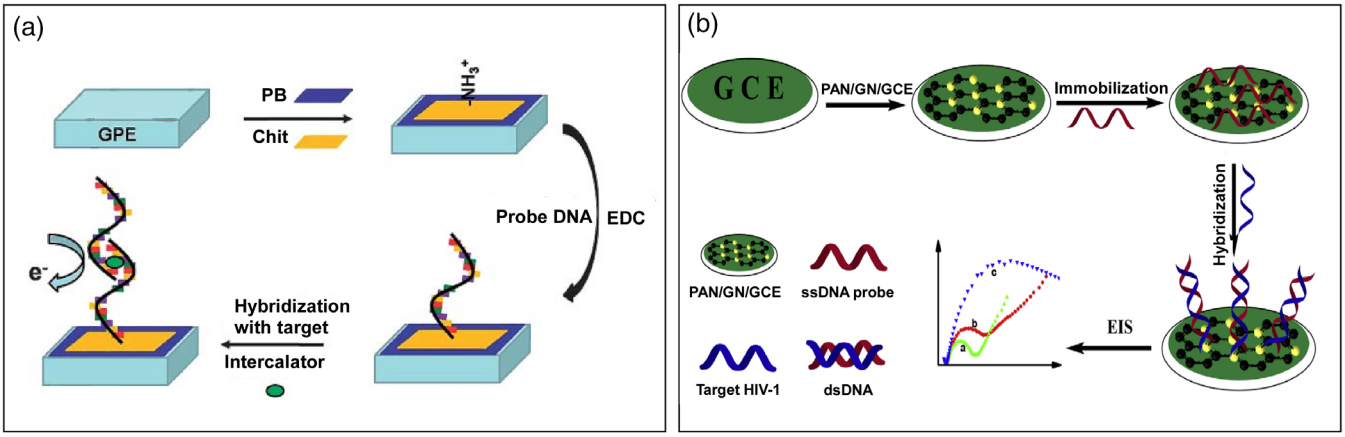

FIGURE 1 DNA probe immobilization via physical adsorption. (a) The ssDNA probes attachment on chitosan coated graphite paste electrode via electrostatic interaction between the negatively charged phosphate backbone of DNA probes and the cationic amino groups of chitosan. (Released with permission from Bo et al. [2011]. Copyright [2011] The Royal Society of Chemistry). (b) The ssDNA probes immobilized on PAN/graphene modified GCE surface via π–π* stacking interaction between DNA bases and PAN/graphene nanocomposite. (Released with permission from Gong et al. [2019]. Copyright [2019] The Chinese ceramic society)

achieved through van der Waals and/or electrostatic interactions between the polymer-coated electrode surface and the DNA strands. Various cationic polymeric materials, such as chitosan, poly-L-lysine, polypyrrole, and polyaniline, have been used for the physisorption of DNA probes onto the electrode surface (Aydemir et al., 2016; Rahman et al., 2015). The formed cationic layers on the electrode surface provide a high surface charge density and excellent biocompatibility.

For instance, Bo et al. (2011) demonstrated the successful immobilization of DNA probes on a chitosan-coated graphene paste electrode utilizing electrostatic adsorption. As shown in Figure 1a, the chitosan, a natural polysaccharide, formed an anime (—NH_2) layer (positively charged at neutral pH) on the electrode surface. The DNA probes then readily attach to the chitosan thin film via the more vital interaction between the negatively charged phosphate backbone of DNA probes and the cationic amino groups of chitosan. This coating process provided a better voltammetric response for complementary DNA binding than ordinary carbon paste electrodes. Polyaniline (PAN) is another widely used conducting polymer in the field of electrochemical sensing due to its excellent properties of high conductivity, chemical durability, and environmental stability. Gong et al. (2019) reported that the DNA probes could bind on the modified electrode surface via π–π stacking interaction between DNA probes and PAN/graphene modified glassy carbon electrodes. By taking PAN's excellent conductivity and the large surface area of graphene, the fabricated biosensor was able to detect target HIV-1 with a low detection limit of 1.0×10^{-16} M (Figure 1b). Beside polymers, nanomaterials have also been used for direct immobilization of DNA probes through noncovalent interaction (Ahour & Shamsi, 2017; Gerbreders et al., 2019).

Although physisorption provides a simple approach for immobilizing DNA probes on the electrode surfaces, the nature of the DNA-surface interaction can cause issues such as nonspecific and weak absorption of DNA probes, high background noise, and electrode fouling (Lichtenberg et al., 2019). Also, the interaction between the adsorbed probe DNA and the electrode randomly along its entire length limits the accessibility of target molecules to some degree, resulting in poor target hybridization efficiency and low detection sensitivity.

2.1.2 | Covalent interaction

Covalent bonding is another effective and commonly used strategy to immobilize DNA probes on the electrode surface. In general, the DNA probes are modified with thiol (—SH) or amine (—NH_2) groups at either their 3′ or 5′ end. These functional groups can form strong covalent coupling with the metal electrode surface itself or specific functional groups coated on the electrode surface (Cai et al., 2003). Although the covalent bonding technique requires further modification of DNA probes and multiple chemical reaction processes, it provides the SAM of DNA probes with high binding strength and excellent stability to prevent DNA probes from desorption during the sensing reaction. Different covalent

coupling chemistries involving various functional groups have been extensively studied to attach DNA probes on the electrode surface. Amine terminated ssDNA is one of the popular probes, which could form covalent bonds with several functional groups, such as carboxyl, aldehyde, isothiocyanate, epoxy, and so on attached on the electrode surface. For example, Han et al. fabricated a DNA biosensor by covalently coupling the NH_2 terminated PNA probes with aldehyde groups on the surface a glassy carbon electrode (GCE). The aldehyde groups were introduced on the electrode surface by electrodeposition of 5-formylindole under weakly acidic conditions. PNA probes then were covalently immobilized on the GCE using condensation pathway of aldimine as shown in Figure 2a (Y. Han, An, et al., 2020; S. Han, Liu, et al., 2020). This covalent coupling method has been extensively employed for DNA probe immobilization (A. Liu et al., 2011a; Q. Wang et al., 2011; B. Xu et al., 2015; L. Zhu et al., 2012). EDC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) is the most popular crosslinker used for covalently coupling substances containing carboxyl groups and amines (NH_2) (Costa et al., 2014; Niu et al., 2017; Settu et al., 2017). As illustrated at Figure 2b, L. Yang et al. (2015), successfully attached the NH_2-DNA probes onto the carboxyl-functionalized graphene oxide/single-CNT modified GCE through EDC/NHS coupling reaction. In another study, D. Wang, Hu, et al. (2013) reported the covalent immobilization of NH_2-DNA probes on electrode by sulfanilamide coupling reaction (Figure 2c). The GCE was first modified with Eriochrome Black T (pEBT) film via electropolymerization of pEBT on the electrode surface. The DNA probes were then grafted on the pEBT layer through the sulfamide coupling reaction between the amino groups in the probe DNA and the free sulfonic groups on pEBT.

Thiol-based SAMs on metals, particularly on Au, have been widely used for covalent binding of DNA probes on electrode due to the strong binding affinity and high stability of the S—Au bond, as well as the easy preparation. Wang et al. introduced a highly sensitive DNA biosensor constructed by covalently binding thiol modified hairpin-like DNA probes on a gold electrode surface. On the other end of the probes, AuNPs, acting as a signal amplification platform, were tethered, which enhanced the electroactive loading and electron transfer kinetics of melamine-Cu^{2+} complex, thereby significantly boosting the electrochemical response of the biosensor (Figure 2d; Q. Wang et al., 2016). Cui et al has used the Au—S covalent interaction method to immobilize methylene blue (MB) modified DNA probes to detect adenosine. (Cui, Hu, et al., 2018; Cui, Li, et al., 2018; Cui, Lu, et al., 2018). Although S—Au coupling can form a strong monolayer of DNA probes on the electrode surface, it cannot prevent the unmodified oligonucleotides from being physiosorbed on the electrode surface, thereby affecting the detection sensitivity of biosensors. To solve this issue, Herne and Tarlov (1997) demonstrated a DNA immobilization strategy that employed a mixed SAM on a gold electrode surface, where the SH-DNA strands acted as detection probes, and mercaptohexanol (MCH) served as a spacer molecule. By adjusting the ratio of these two elements, this design not only provides control of the density of DNA probes and helps the DNA probes stand upright on the surface, but also repels nonspecific adsorption of irrelevant DNA. By adopting the same strategy, Zhang et al. reported a novel electrochemical DNA sensor with a mixed SAM of SH-DNA capture probes (CPs) and oligo (ethylene glycol) (OEG)-terminated thiols on the gold surface (Figure 2e; J. Zhang et al., 2008). They found that the mixed SH-DNA/OEG exhibited a nonfouling feature and allowed facile electron transfer across the monolayer than MCH. Based on this SAM platform, they developed a sandwich-type electrochemical sensor for the sequence-specific detection of DNA targets with a detection limit of 1 pM.

In sensor design, various nanomaterials, such as metal nanoparticles, QDs, magnetic nanoparticles, CNTs, graphene, and so on have been used to modify the working electrode, which can increase the surface area of electrodes and therefore the number of immobilized DNA probes. Among them, AuNPs have received immense interest because of their large surface area to-volume ratio with facile immobilization of DNA probes through the strong affinity of covalent Au—S bonds. For example, J. Wang et al. (2014) utilized AuNP decorated graphene oxide sheets to modify a GCE to covalently immobilize DNA probes on the hybrid nanocomposite surface via the formation of Au—S bond (Figure 2f). Similarly, C. Xu et al. (2014) fabricated a DNA biosensor based on the layered structure of CuS-graphene nanocomposite and AuNPs. The graphene served as two-dimensional conductive skeleton support for CuS, and AuNPs were used to immobilize SH-DNA probes. This composite provides a stable and conductive interface for electrochemical DNA detection with a low detection limit of 0.1 pM (Figure 2g). In 2020, Cui et al. (2020) reported the fabrication of a cathodic photoelectrochemical sensor based on the integration of covalent organic polymer with palladium nanoparticles to immobilize aptamer via palladium-sulfur chemistry for the sensitive detection of cancer cells. L. Yang et al. (2021) developed an electrochemical biosensor for hydrogen peroxide and microRNA detection. First multi-walled CNTs were doped with electroactive Prussian blue. Then the nanocomposite polypyrrole/multi-walled CNTs/Prussian blue arrays were electrochemically synthesized and deposited on the GCE surface. Finally, the amino-functionalized DNA CP were immobilized on the nanocomposite via the covalent coupling reaction between the amino groups and carboxyl groups of the nanocomposite (Figure 2h). This design guaranteed good electrical conductivity and a high

surface-to-volume ratio for miRNA-24 biosensing. Jahanbani and Benvidi (2016) also designed a label-free DNA biosensor using a magnetic bar carbon paste electrode, which was modified with Fe_3O_4/reduced graphene oxide as a nanocomposite and 1-pyrenebutyric acid-*N*-hydroxysuccinimide ester (PANHS) as a linker for detection of DNA. DNA probes were immobilized on the electrode through the coupling reaction between the succinimide ester group on PANHS and the amine group in the probe DNA. With this strategy, they developed low-cost and label-free sensors without NHS/EDC's need to activate the carboxylic groups on the surface.

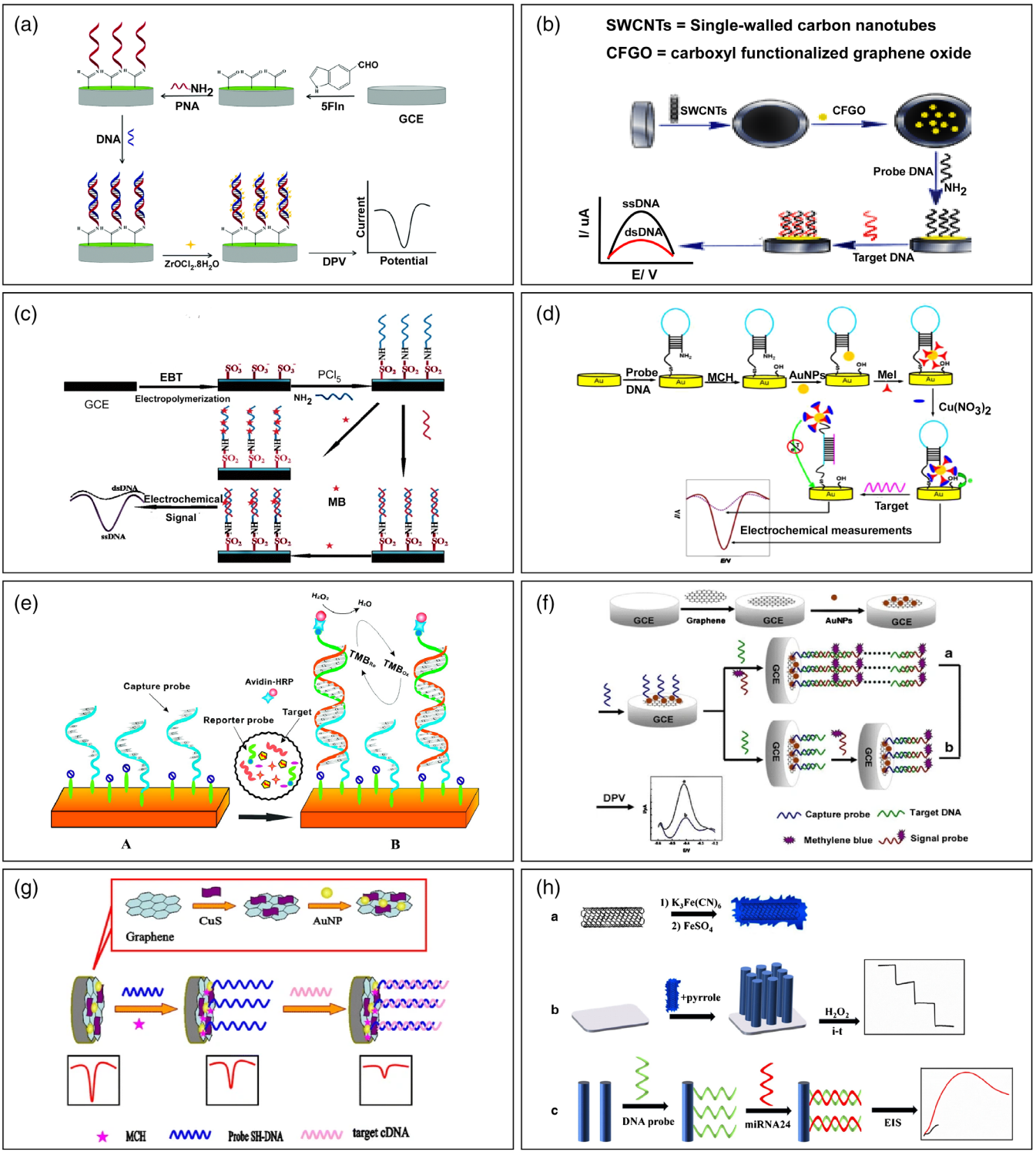

FIGURE 2 Legend on next page.

2.1.3 | Biotin–avidin/streptavidin interaction

Besides the chemical interactions described in the previous sections, the biomolecular interaction of biotin–avidin/streptavidin can also be used to immobilize DNA probes on electrode surfaces in the fabrication of electrochemical biosensors (Smith et al., 2006). Because of its small size and good stability, biotin rarely interferes with the function of labeled molecules while enables the formation of a powerful complex with avidin. In different pH values, temperatures, organic solvents, and other denaturants, the avidin—biotin bond formation is very fast and stable (Jain & Cheng, 2017). In addition, avidin is a protein containing four identical biotin-binding sites, which provides the opportunity to connect multiple biotinylated DNA probes. Many studies have used these advantages for nucleic acid detection.

G. Liu et al. (2008) reported an enzyme-based electrochemical DNA biosensor for sequence-specific detection of nucleic acids, prepared by immobilizing the biotinylated stem-loop DNA probes on the electrode surface using the biotin−avidin bridge (Figure 3a). The gold electrode was first functionalized with a mixed SAM composed of thiolated 11-MUA/11-MU, where 11-MUA provided carboxylic conjugation sites and 11-MU served as a surface passivation agent. After the densely packed SAM was formed, amine-modified biotin was attached to the conjugation site via covalent bonding between carboxylic acid and amine groups with EDC/NHS help. Afterward, streptavidin was coupled to the surface of the biotinylated gold electrode. Finally, the biotin modified stem-loop DNA probes were attached on the surface via avidin–biotin interaction. This fabricated biosensor detected DNA targets at the femtomolar level with excellent single-base mismatch differentiation ability. Chung et al. also developed biosensor for influenza virus detection based on the similar avidin attachment principle. In the other study, streptavidin was directly attached on the 1,1′-carbonyldiimidazole (CDI) modified electrode surface for human papilloma virus-16 E7 gene detection, as illustrated in Figure 3b (J. Lin et al., 2019). CDI, a chemical linker used for conjugating amino acids during peptide synthesis, can couple the amine groups in streptavidin to the sensing surface's hydroxyl groups. This sensor was able to detect the target of 1 attomole with high selectivity. The same strategy has also been used to detect cardiac troponin I (cTnl) biomarker (C. C. Zhang, Sun, et al., 2020). Furthermore, Yesil et al. (2016) reported using poly(L-glutamic) acid films coated on the pencil graphite electrode surface to anchor avidin for immobilization of biotinylated probe DNA. The attachment of DNA probes is simple via avidin–biotin interaction on the electrode surface, however, it needs multiple and different chemical reactions to fix the avidin on varied electrode surface, as well as the passivation procedure to avoid the nonspecific physical adsorption (Nimse et al., 2014).

2.2 | Indirect probe immobilization using DNA nanostructure as scaffold

In this strategy, ssDNA probes are not directly immobilized on the electrode surface, instead of attached on DNA nanostructure platforms, which are then immobilized on the electrode surface by either physical adsorption or covalent interaction. It is well known that the density and upright orientation of DNA probes on the electrode surface play an important role in determining the biosensor's detection capabilities (Pei et al., 2013). The accessibility of target molecules will be limited with the dense layer of probes due to electrostatic repulsion and steric hindrance. In contrast, the

FIGURE 2 DNA probe immobilization through covalent bonding. (a) Amino group modified PNA probes conjugate with aldehyde groups on the surface of a GCE. (Released with permission from S. Han, Liu, et al. [2020]. Copyright [2020] The Royal Society of Chemistry). (b) Attachment of the ssDNA probes through aminos group on DNA probes and carboxyl groups on carboxyl-functionalized graphene oxide modified electrode. (Released with permission from L. Yang et al. [2015]. Copyright [2015] The Royal Society of Chemistry). (c) The ssDNA probes grafted on the pEBT layer through the sulfamide coupling reaction between the amino groups on the probe DNA and the free sulfonic groups on pEBT. (Released with permission from L. Wang, Liao, et al. [2013]. Copyright [2013] Springer-Verlag Wien). (d) The thiol-modified hairpin DNA probes directly bound to the gold electrode surface through Au–S interaction. (Released with permission from Q. Wang et al. [2016]. Copyright [2016] Scientific Reports). (e) Thiol-modified ssDNA probes and oligo (ethylene glycol)-terminated thiols self-assembled on the gold electrode surface generating nonfouling sensing surface. Copyright [2008] The American Chemical Society). (f) Thiol-modified ssDNA probes immobilization on GCE modified with AuNP decorated graphene oxide sheets via Au–S. (Released with permission from J. Wang et al. [2014]. Copyright [2014) Springer-Verlag Wien). (g) SH-DNA probes assembled on GCE fabricated with CuS-graphene nanocomposite and AuNPs through Au–S linkage. (Released with permission from C. Xu et al. [2014]. Copyright [2014] Springer-Verlag Berlin Heidelberg). (h) The NH_2-modified ssDNA probes immobilized on the polypyrrole/multi-walled carbon nanotubes/prussian blue nanocomposite modified GCE via covalent coupling between the amino groups of probes and the carboxyl groups of the nanocomposite. (Released with permission from L. Yang et al. [2021]. Copyright [2021] Springer Nature)

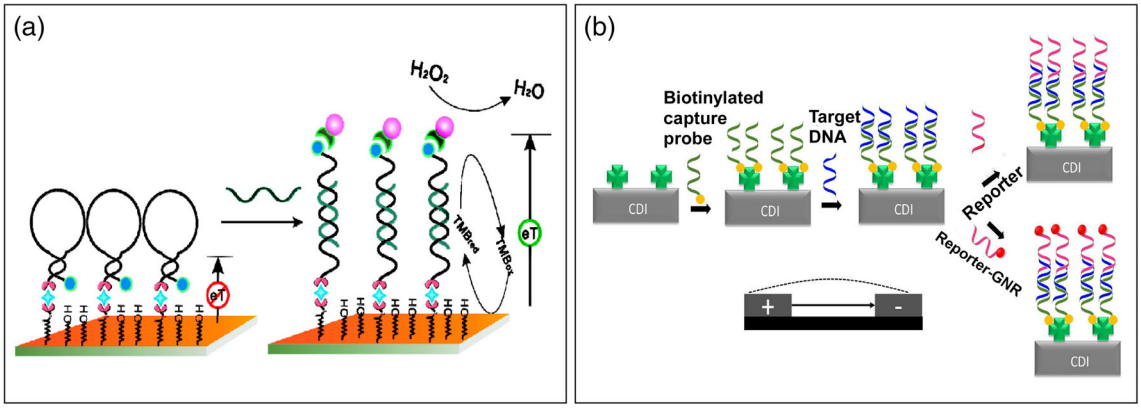

FIGURE 3 Biotin–avidin/streptavidin interaction assisted immobilization. (a) Biotin-modified hairpin DNA probes immobilized on an avidin-modified electrode surface via the biotin–avidin conjugation. (Released with permission from G. Liu et al. [2008]. Copyright [2008] American Chemical Society). (b) Biotinylated capture probes immobilized on the CDI surface modified with streptavidin through the biotin-streptavidin interaction. (Released with permission from J. Lin et al. [2019]. Copyright [2019] Elsevier)

sparsely dispersed DNA probes will decrease the hybridization yield between probes and targets because of the probes' nonspecific physical adsorption on the electrode surface. Although various methods, such as the using of mixed SAM and different probe designs (hairpin-loop, triple-stem, and pseudoknot probes), have been explored, novel strategies are still highly needed to improve the probe immobilization technique, increase the recognition capability of target-probe, thus enhance the sensitivity of the biosensor (Kjällman et al., 2008; Y. Liu et al., 2013; White et al., 2008; Y. Xiao et al., 2007; Zhou et al., 2011).

The development of DNA nanotechnology initiated by Dr. Seeman shined the light on the bottom-up nanofabrication, created numerous artificial shapes at the nanometer scale, including DNA nanoobjects, 1D linear arrays, 2D, and 3D crystalline structures (Liu et al., 2011b; W. Liu et al., 2016; Seeman & Sleiman, 2017; J. B. Zheng et al., 2009). The application of those nanostructures fabricated by DNA hybridization has been explored in biosensing, drug delivery, disease therapy, manufacturing, and material science, owing to their diversity, biocompatibility, and easy attachment of functional materials (Zahid et al., 2013; F. Zhang et al., 2014). Among those structures, the simplest design of DNA tetrahedron has been extensively used to immobilize DNA probes on the surface of electrochemical probes, which Dr. Fan's group first proposed in 2010 (Pei et al., 2010). In their design, the 3D tetrahedron was attached to the Au surface via three thiol groups, one for each vertex, while the DNA probe was attached to the fourth vertex (Figure 4a). This strategy contributed to the rigidity and upright orientation of the probes and was able to control the distance between adjacent probes, which is critically important for efficient target hybridization. They demonstrated that the DNA tetrahedron-assisted biosensor exhibited a 250-fold improvement in sensitivity (~1 pM) compared with that of linear DNA probes employed the similar horseradish peroxidase (HRP)-based sandwich sensing system. Later, Lin et al. studied the effects of different sizes of tetrahedral DNA nanostructures on the kinetics and thermodynamics of target hybridizations (Figure 4b; M. Lin et al., 2015). They found that hybridization time was shortened, and the hybridization efficiency was increased by optimizing the size of tetrahedron, which led to attomolar sensitivity with polymeric enzyme amplification. Since then, different types of electrochemical biosensors have been developed based on the tetrahedron design (Y. L. Huang et al., 2017; J. L. Wang, Dong, et al., 2019; Y. H. Wang, He, et al., 2019; X. Wang, Niu, et al., 2019; L. Xu et al., 2018; D. W. Zeng et al., 2017). To simplify the sensing process, C. C. Li, Hu, Lu, & Zhang (2018) constructed a novel DNA tetrahedron-based electrochemical sensor to detect biomolecules directly. In their system, two additional strands were extended from the two vertices of the tetrahedron (Figure 4c). The top one was used as a probe strand, and the bottom one acted as an assistant strand. This modification allows the flexible addition of the recognition elements that specifically bind the analyte between the probe and assistant strands. This sensor has been used to detect DNA directly in whole blood samples. Recently, Fan et al. (2021) developed an electrochemiluminescence biosensor to detect the RNA-dependent RNA polymerase gene of SARS-CoV-2 known as RdRp-COVID, in which DNA tetrahedron served as robust scaffolds of DNA probes and entropy-driven reaction as signal amplifiers in the diagnosis of COVID-19 with a limit of detection down to 2.67 fM.

Besides the DNA tetrahedron, DNA origami nanostructures have been widely used for nonelectrochemical biosensing because of their structural diversity, larger surface area, and capability to provide hundreds of

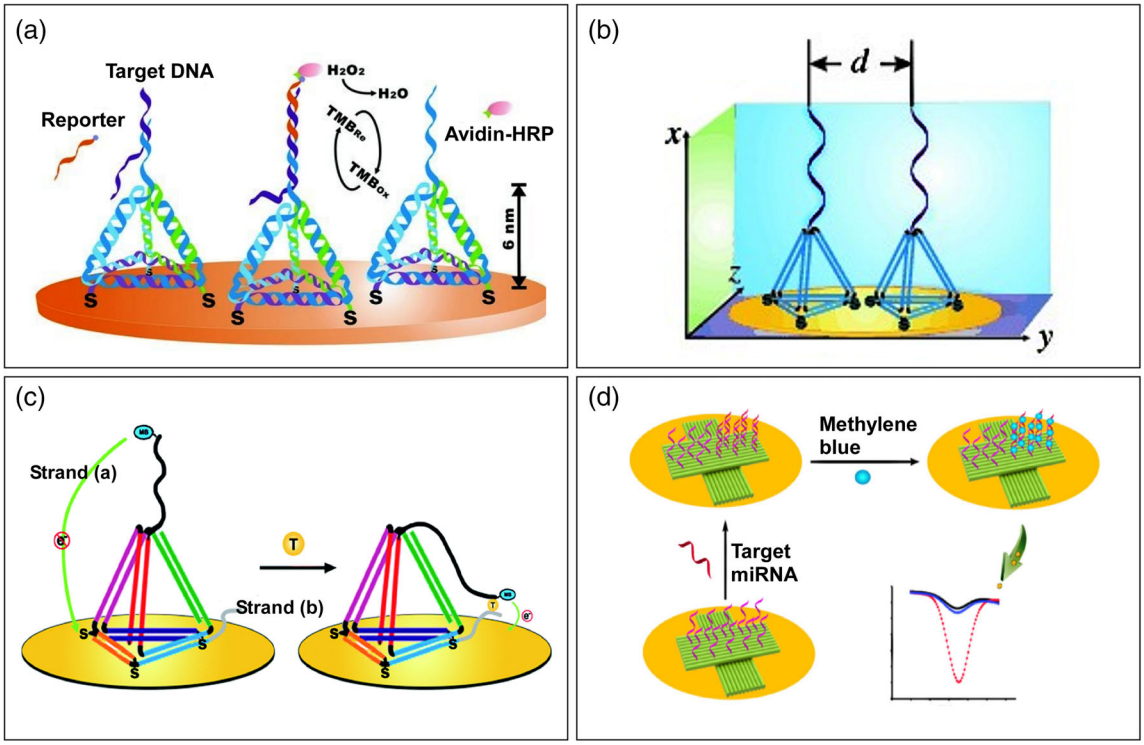

FIGURE 4 DNA nanostructure-assisted DNA probe immobilization. (a) The ssDNA probe was attached on the top vertex of DNA tetrahedron on electrode. (Released with permission from Pei et al. [2010]. Copyright [2010] Wiley-VCH). (b) Kinetic and thermodynamic studies of differently sized DNA tetrahedron on hybridization and sensitivity of the DNA sensor. (Released with permission from M. Lin et al. [2015]. Copyright [2015] Wiley-VCH). (c) Novel DNA tetrahedron with dual probe for target detection in whole blood samples. (Released with permission from Chao et al. [2016]. Copyright [2018] The Royal Society of Chemistry). (d) The cross-shaped DNA origami incorporated with multiple ssDNA capture probes for miRNA target. (Released with permission from S. Han et al. [2019]. Copyright [2019] American Chemical Society)

staple strands for modification at the rationally controlled distance on the nanometer scale (Subramanian et al., 2011; J. F. Zhu et al., 2013). Ke et al. (2008) developed a label-free microRNA sensor using rectangular DNA origami as a platform. They extended the staple strands at predefined positions with the sequences designed to bind microRNA segments of biological interest. The hybridization between the staple extensions and the microRNA targets created local protrusions from the origami surface, which could be detected by atomic force microscopy (AFM). Similarly, Subramanian et al. fabricated a direct visual readout of the target nucleotide in the probe sequence by combining the techniques of AFM and DNA origami. The origami platform contains graphical representations of the four nucleotide alphabetic characters, A, T, G, and C. The symbol containing the test nucleotide identity will vanish in the presence of the probe. S. Han et al. (2019) for the first time, developed a novel, label-free, and amplification-free miRNA electrochemical biosensor for the miRNA-21 detection, using DNA origami as a probe DNA platform (Figure 4d). Compared with DNA tetrahedron, the cross-shaped DNA origami sensing platform could provide a large number of free-standing DNA probes to capture target nucleic acids with enhanced probe accessibility and high sensitivity, as well as the prevention of physical adsorption on electrode surface, resulting in a miRNA detection limit of 80 fM. DNA origami structures provide a promising strategy to further enhance the design and sensitivity of electrochemical biosensors. Table 1 listed the commonly used probe immobilization techniques.

3 | SIGNAL AMPLIFICATION STRATEGIES

Electrochemical biosensors are based on converting biomolecule recognition events into detectable electrical signals on the biosensor surface. The hybridization between DNA/RNA targets and the probes immobilized on the electrode

TABLE 1 Direct immobilization of DNA probes

Methods	Principle	Materials/reagents on electrode surface	Analyte	Limit of detection	References
Direct immobilization of DNA probes					
Physical adsorption	Van der Waals and/or electrostatic interactions between the polymer-coated electrode surface and the DNA strands	Chitosan	DNA	1.58×10^{-11} mol dm^{-3}	(Bo et al., 2011)
		Polyaniline (PAN)	HIV-1 DNA	1.0×10^{-16} M	(Gong et al., 2019)
		Poly (indole-6-carboxylic acid)	DNA	2.3×10^{-18} mol/L	(J. Yang, Gao, et al., 2019; B. Yang, Zhang, et al., 2019)
		Graphene oxide	DNA	4.3×10^{-11} M	(Ahour & Shamsi, 2017)
Covalent bond formation	DNA probes are modified with thiol (—SH) or amine ($—NH_2$) which form strong covalent coupling with the metal electrode surface itself or specific functional groups coated on the electrode surface	Bare Au	Adenosine	90.8 pM	(Cui, Hu, et al., 2018; Cui, Li, et al., 2018; Cui, Lu, et al., 2018)
		Aldehyde groups	DNA	0.81 pM	(Y. Han, An, et al., 2020; S. Han, Liu, et al., 2020)
		Carboxyl group	*Thermolabilehemolysin* gene	7.21×10^{-14} mol L^{-1}	(L. Yang et al., 2015)
		Ester group	BRCA1 5382 insC gene	2.8 × 10–19 mol/L	(Jahanbani & Benvidi, 2016)
Biotin-avidin/ streptavidin affinity interaction	Biotin-modified DNA is immobilized on avidin/ streptavidin modified electrode surface by their specific interaction.	Avidin	DNA	0.162 fM	(G. Liu et al., 2008)
			Mycobacterium tuberculosis DNA	1.3 nM	(Yesil et al., 2016)
		Streptavidin	Human papilloma virus-16 E7 gene	1 aM	(J. Lin et al., 2019)
			Cardiac troponin I	1 fM	(C. C. Zhang, Sun, et al., 2020)
DNA nanostructure as probe scaffold					
Covalent bond interaction/ physical adsorption	DNA nanostructure platform is used as scaffold to hold DNA probes. The ssDNA probe on nanostructure is then immobilized on the electrode surface usually by physical adsorption or covalent interaction	DNA tetrahedron	DNA	100 pM	(Pei et al., 2010)
			DNA	300 fM	(C. Li, Hu, Lu, Mao, et al., 2018)
		DNA origami	miRNA-21	80 fM	(S. Han et al., 2019)

surface can trigger the change of electrochemical behavior by monitoring the redox peak current signals from the electroactive bases of DNA or from external hybridization indicators (Hai et al., 2020).In order to improve the sensitivity and specificity of electrochemical biosensors and make them suitable for effective analysis of lower-level of biomarkers in clinical samples, signal amplification strategies, such as electrochemical active substances, enzyme catalysis, hybridization chain reaction (HCR), rolling circle amplification (RCA), and nanomaterial-supported amplification, are commonly employed to enhance the signal of the DNA/RNA target and probe binding reaction (Y. X. Chen et al., 2018; Y. Liu, Liu, et al., 2018; S. Liu, Yang, et al., 2018). These techniques could reduce background noise and enhance the output signal of biosensors.

3.1 | Enzyme-based amplification

Due to the excellent catalytic activity and substrate specificity, enzymes have been widely used as signal amplifiers in electrochemical biosensors, which can create multiple-fold-higher redox-active products, thereby significantly enhancing the redox current at the electrode surface. Commonly used enzymes include HRP, glucose oxidase (GOx), alkaline phosphatase (ALP), and so on.

Ilkhani and Farhad (2018) developed a sensitive electrochemical biosensor for Ebola virus DNA detection based on an ALP-supported signal amplification strategy (Figure 5a). The thiolated DNA CPs immobilized on the electrode surface were first hybridized with biotinylated target DNA strands. Then, streptavidin modified ALP enzymes were added into the system via streptavidin-biotin conjugation. Differential pulse voltammetry (DPV) measurement technique was utilized to detect the oxidation signal of the enzymatically produced 4-aminophenol. The fabricated biosensor was able to detect the Ebola virus DNA with a detection limit as low as 4.7 nM. Xia et al. (2015) reported an ultrasensitive electrochemical method for miRNA detection, which is also based on ALP amplification followed by electrochemical–chemical–chemical (ECC) redox cycling (Figure 5b). The enzyme attached to DNA probes catalyzed the production of thousands of ascorbic acid (AA), which increased ECC redox cycling efficiency, leading to a detection limit of 0.2 fM, which is even lower than that obtained using HRP or GOx amplification. Besides using the biotin modified DNA probes or targets for direct attachment of streptavidin modified enzymes, a sandwich hybridization method is also commonly used in enzyme-based electrochemical detection. For example, Dong et al. (2015) used this strategy to detect the target DNA of the influenza A (H7N9) virus by using avidin-HRP as the signal amplifier and DNA tetrahedral structures as probe templates (Figure 5c). After the hybridization of the target and probe DNA, the biotin modified reporter DNA bound on the captured target sequence, and then avidin-HRP was introduced to produce an amperometric signal through the oxidation of 3, 3′, 5, 5′-tetramethylbenzidine (TMB) during the enzymatic degradation of H_2O_2 by HRP. The biosensor could reach a 100 fM detection limit for target sequences and be able to distinguish single-base mismatch oligonucleotides.

To further improve the sensitivity, enzymatic reactions have been combined with other amplification processes like cycling reaction, multienzyme labels, or functional nanoparticles (J. Guo et al., 2018; J. Yang, Gao, et al., 2019; B. Yang, Zhang, et al., 2019; L. Zhang, Deng, et al., 2019; H. Zhang, Fan, et al., 2019). For instance, Hu et al. (2014) developed an electrochemical biosensor to detect DNA sequence of *Bacillus subtilis* by coupling target-induced strand displacement and nicking endonuclease signal amplification strategy together (Figure 5d). The strand displacement reaction was initiated by target hybridization. When target DNA hybridized with part of the CP, the ferrocene modified signal probe (SP) was displaced, causing the ferrocene modified end to collide to the electrode surface, thereby increasing the electron transfer. Meanwhile, the nicking endonuclease bound to and nicked the recognition position causing more and more ferrocene probes to quickly approach the electrode surface to receive significant current. After nicking, the target DNA was liberated from the CP. The free targets then underwent multiple hybridization cycles, resulting in a significant current signal even with a low concentration of the target. This design makes the biosensor is capable of achieving a lower detection limit down to 0.08 fM. Shuai et al. (2016) employed a similar method by using SA-ALP signal amplification along with catalyzed hairpin assembly target recycling to detect miRNA.

Though the enzyme-supported signal amplification methods are extensively used in biosensor devices, some disadvantages still exist, such as enzyme stability, sensitivity to the sensing environment, and time-consuming preparation processes. Therefore, many nanomaterial-based enzymes have been developed, which have the advantages of easy preparation, high activity to catalyze, and low cost (X. Li et al., 2020). For example, Tian et al. (2020) reported streptavidin functionalized $CoFe_2O_4$ nanozyme as an electrochemical signal amplifier for sensitive detection of kanamycin (Kana), with a detection limitation of 0.5 pM. Cui et al. fabricated a mimic peroxidase and bismuth sulfide nanorod-based

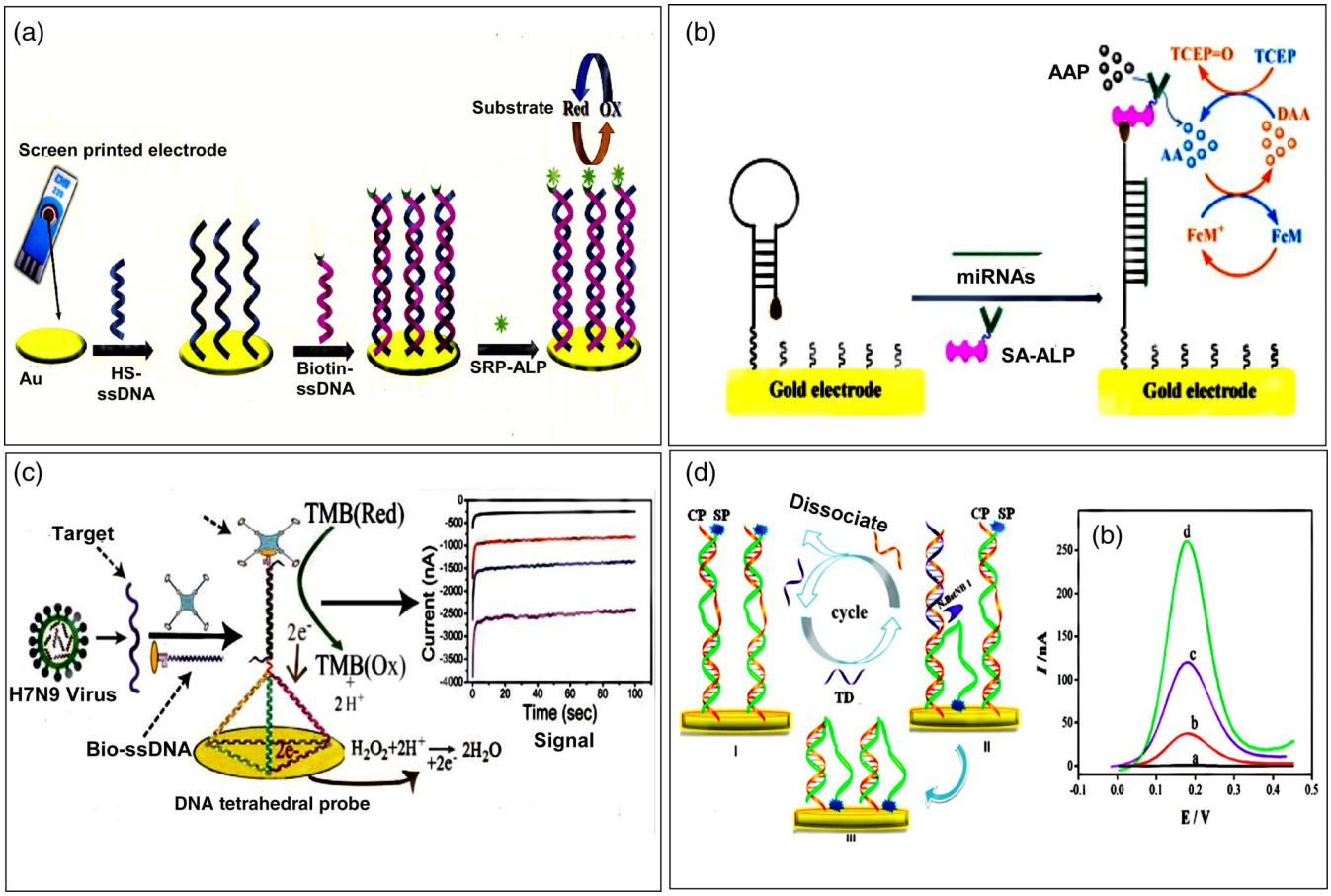

FIGURE 5 Enzyme-based signal amplification; (a) The biotinylated target DNA strands hybridized with capture probes, then bound with streptavidin modified ALP enzymes which catalyze the oxidation and reduction of substrate giving amplified electrochemical signal. (Released with permission from Ilkhani and Farhad [2018]. Copyright [2018] Elsevier Inc.). (b) The ALP enzyme attached to DNA probes after target hybridization catalyzing the production of ascorbic acid (AA), which increased ECC redox cycling efficiency, leading to higher signal amplification. (Released with permission from Xia et al. [2015]. Copyright [2015] Elsevier B.V.). (c) Hybridization of the target DNA on tetrahedral probe followed by biotin-modified reporter DNA addition. Then the avidin-HRP signal amplifier attached to reporter probe giving the electroactive signal. (Released with permission from Dong et al. [2015]. Copyright [2015] American Chemical Society). (d) The nicking endonuclease nicked the recognition position causing ferrocene probes to quickly approach the electrode surface. Meanwhile the target DNA was liberated from the capture probe which can reduce multiple hybridization cycles, resulting in a significant current signal. (Released with permission from Hu et al. [2014]. Copyright [2014]. The American Chemical Society)

photoelectrochemical biosensor for the sensitive detection of polynucleotide kinase (Cui, Hu, et al., 2018; Cui, Li, et al., 2018; Cui, Lu, et al., 2018). There are several comprehensive reviews of recent progress on nanozymes and their applications. (Mahmudunnabi et al., 2020). Some of nanoparticles-based enzymes will be further discussed in nanomaterial-based signal amplification part.

3.2 | DNA enhanced signal amplification

Nucleic acid-based amplification technology is another promising signal amplification strategy to improve the detection sensitivity of various targets for electrochemical biosensors. In this technique, enzymes and nucleic acid cooperatively elongate the DNA chain or helps to cleave DNA at a specific location affecting the corresponding electrochemical signal. Many enzymes, such as hemin/G-quadruplex D, duplex-specific nuclease (DSN), exonuclease, endonuclease, and polymerase have been employed in signal amplification. (Z. Chen et al., 2019; Fan et al., 2021; J. Y. Huang et al., 2018; Miao et al., 2018; J. L. Wang, Dong, et al., 2019; Y. H. Wang, He, et al., 2019; X. Wang, Niu, et al., 2019; Wu et al., 2020; You et al., 2018; M. Zhang et al., 2018; L. Zhang, Deng, et al., 2019; H. Zhang, Fan, et al., 2019). Enzymes like

endonuclease (Ji et al., 2017) helps in cleaving the DNA at a predesigned position, whereas polymerase assists in the elongation of DNA to help the signal increments. In this review, we will discuss enzyme-assisted and nonenzymatic DNA-based signal amplification methods.

3.2.1 | Rolling circle amplification

RCA is an isothermal nucleic acid amplification technique, which has become an attractive signal amplification tool for electrochemical biosensing (Fu et al., 2020; F. Xiao et al., 2020). In this method, with the assistance of polymerase catalysis, a short DNA/RNA primer is extended by using a circular DNA as the template to form long ssDNA/RNA. RCA is a rapid technique that can produce many duplications of circular templates within 1–3 h at the constant temperature without the requirement of expensive equipment (Mohsen & Kool, 2016). Therefore, RCA has been rapidly developed and applied in molecular biology, materials science, and medicine to enhance the detection sensitivity of various targets, including DNA, RNA, proteins, pathogens, and anticancer drugs (L. Xu et al., 2021) .

Miao et al. (2015) prepared a DNA tetrahedron decorated gold electrode for high-precision detection of miRNA, based on RCA signal amplification strategy (Figure 6a). The DNA tetrahedron immobilization was realized with thiols on three vertexes through Au–S interaction on the gold electrode. The linear pedant sequence of ssDNA on the fourth vertex was used to capture target miRNA. The miRNA further hybridized with the RCA primer-probe forming a sandwich structure on the surface, which initiated the RCA reaction with the assistance of a circular DNA template and phi29 DNA polymerase to produce a long ssDNA. The attachment of a large number of silver nanoparticle (AgNP)-labeled SPs on the long ssDNA provided significant electrochemical signals, which led to a detection limit as low as 50 aM and high selectivity. Wang et al. also developed an ultrasensitive miRNAs biosensor based on a similar RCA method. Upon opened by the target miRNAs, the LNA-molecular beacon (MB) CP was extended to a long ssDNA through the RCA reaction. The obtained RCA product contains thousands of repeated sequences, which allowed for hybridization with the QDs-modified detection probes. This strategy could enhance the target binding efficiency and discriminate mismatched sequences, therefore resulting in an ultra-low detection of 0.32 aM (D. Wang, Hu, et al., 2013). Signal amplification strategies of RCA along with DNA–AuNPs probe was used by D. Zhu et al. (2014) to improve the sensitivity of Salmonella gene detection. The long DNA sequences synthesized by RCA provided attachment for a large number of DNA–AuNPs probes to enhance the recognition. Finally, the resulting AuNPs-tagged RCA product was linked to SA-ALP producing enzymatic electrochemical signal readout. This signal amplification strategy provided ultrasensitive platform for the detection of *Salmonella*.

To further improve RCA-based biosensing strategies, Zhou et al. (2020) proposed a cascade signal amplification approach for detecting 16S rRNA gene from *Pseudomonas aeruginosa* by combining the RCA reaction with multipedal

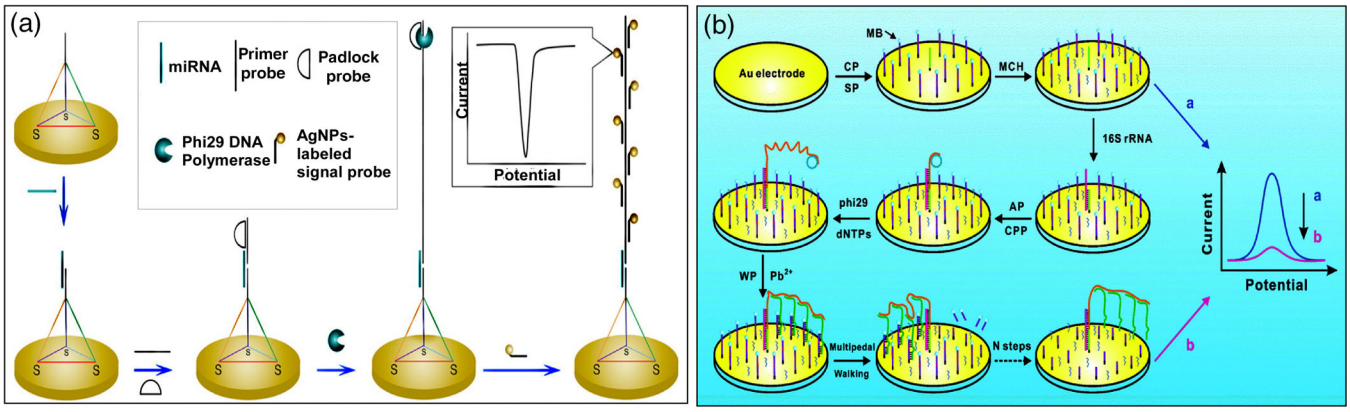

FIGURE 6 Rolling circle-based signal amplification. (a) Partially hybridized target miRNA on the tetrahedral probes further hybridized with the RCA primer that initiated the RCA reaction producing a long ssDNA which could attach many AgNP-labeled signal probes providing significant electrochemical signals. (Released with permission from Miao et al. [2015]. Copyright [2015] American Chemical Society). (b) Target 16S rRNA first hybridized with the capture probe followed by circular padlock probe, which triggered the RCA reaction and released many repeated sequences partially complementary with the walker probes, leading to the formation of multipedal DNA walker. (Released with permission from Zhou et al. [2020]. Copyright [2020] The Royal Society of Chemistry)

DNA walker strategies (Figure 6b). The gold electrode surface was modified with methylene blue (MB)/SH-modified SP and SH-modified CP. In order to avoid nonspecific adsorption, the gold electrode surface was passivated with 6-MCH. Auxiliary probe was added to the Au electrode surface after target 16S rRNA hybridized with the CP followed by addition of a circular padlock probe, phi29 polymerase, and dNTPs, which triggered the RCA reaction and released many repeated sequences partially complementary with the walker probes, leading to the formation of multipedal DNA walker. With the assistance of Pb^{2+}-dependent DNAzyme, the SP is cleaved, resulting in the reduction current peak of MB. The fabricated electrochemical biosensor exhibits superior analytical capability for *P. aeruginosa*. A detection limit of 10 CFU per ml shows great potential applications in disease diagnosis, food safety, and environmental detection (Sarkar et al., 2017).

3.2.2 | Surface-initiated enzymatic polymerization

Surface-initiated enzymatic polymerization is one of the signal amplification methods using terminal deoxynucleotidyl transferase (TdT). TdT is a template-independent DNA polymerase that can catalyze the binding of deoxynucleotides (dNTP) to the 3′hydroxyl end of double-stranded DNA or ssDNA. Unlike other DNA polymerase, TdT does not need a specific DNA template, which is a simple, direct, and cost-effective strategy for ssDNA synthesis (J. Guo et al., 2018). An ultrasensitive electrochemical biosensor was developed by Lang et al. (2020) for the specific recognition of cTnl, a recognized biomarker for diagnosing acute myocardial infarction, which is based on the assists of TdT-mediated signal amplification. In the presence of cTnl, probe 1 (P1) was released from P1/aptamer complex and hybridized with probe 2 (P2) on the electrode surface, forming 3′-OH of DNA. In the presence of TdT and dTTP, TdT catalyzed the formation of poly T extension to the P1 end. Then, methylene blue (MB)-poly A bound with the extended poly T structure and generated an amplified electrochemical signal. The detection limit can be as low as 40 pg ml^{-1} (Figure 7a).

For the further improvement of the sensitivity of the biosensors, various signal amplification strategies, such as redox cycling, nanomaterial-assisted methods, have been incorporated with TdT. In 2019, Cui et al. developed a label-free and immobilization-free electrochemical magnetobiosensor to quantify 5-hydroxymethylcytosine (5-hmc) in genomic DNA by employing a dual signal amplification strategy coupled with TdT and Ru(III) redox cycling. The dsDNA with the biotin incorporated on 5-hmc site specifically attached to the streptavidin-modified magnetic beads (Figure 7b). The TdT enzymatically catalyzed the elongation of DNA on the 3′-OH dsDNA site. Then Ru(III) binds on the phosphate groups of DNA via electrostatic interaction, giving the current signal. The magnetic beads with all those modifications attached to the screen-printed carbon electrode surface can trigger the recycled chemical reaction with magnetic field help. This biosensor exhibits high sensitivity with a detection limit of as low as 9.06 Fm and successfully detects 5-hmc in live cells. Similarly, Hao et al. (2017) integrated the TdT with nanoflowers prepared from C60 fullerene and methylene blue (C60/MB) for ultrasensitive detection of prostate specific antigen (PSA). The TdT assists in increasing the loading of MB on the nucleic acid whereas C60/MB nanoflowers improve the electron transfer. The sensor was reported to have a wide linear range that extends from 15 pg ml^{-1} to 8 ng ml^{-1} of PSA with a limit of detection 1.7 pg ml^{-1}.

3.2.3 | Hybridization chain reaction

HCR is another effective isothermal enzyme-free reaction. A ssDNA initiator triggers a cascade of recognition and hybridization events between two DNA hairpin species containing complementary DNA sequences, resulting in a long ssDNA with tens to hundreds of repeated DNA sequences until the hairpin supply is exhausted (Bi et al., 2017). Unlike other isothermal signal amplification techniques, HCR avoids the drawbacks of the high cost of enzymes and complicated experimental procedures, making it more reliable for practical applications. Due to the high simplicity, stability, and versatility, HCR-based electrochemical sensors have drawn attractive attention (Q. Guo et al., 2020; Liang et al., 2018) .

Yuan et al. (2017) developed an electrochemical biosensor for simultaneous detection of miRNA-141 and miRNA-21 based on target-triggered HCR amplification and two different magnetic NP probes (Figure 8a). In the presence of targets, the thiol-modified hairpin CPs hybridized with miR-141 and miR-21 on a gold electrode, respectively, leading to a conformation change of hairpin probes, which subsequently triggered the HCR to generate a series of binding sequences of magnetic nanoprobes. A large number of the resulting HCR products produces

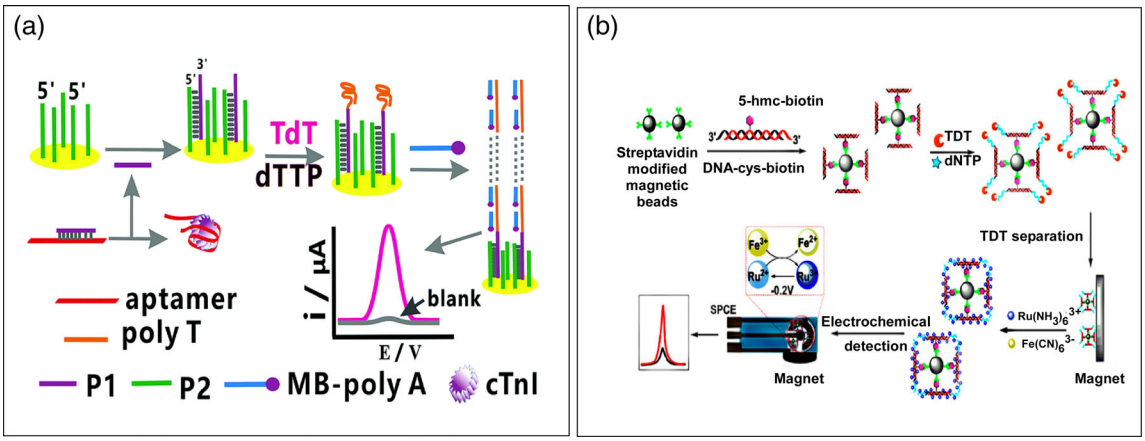

FIGURE 7 Surface-initiated enzymatic polymerization-based signal amplification. (a) An electrochemical sensing platform for the detection of cTnI based on aptamer recognition and signal amplification assisted by TdT. (Released with permission from Lang et al. [2020] Copyright [2020] The Royal Society of Chemistry). (b) Label-free and immobilization-free electrochemical magnetobiosensor for sensitive detection of 5-hydroxymethylcytosine in genomic DNA. (Released with permission from Cui, Wang, et al. [2019] Copyright [2018] 8 American Chemical Society)

increased the capture capability of magnetic nanoprobes, which giving rise to the dual signal amplification with great sensitivity. To further improve the sensitivity, Lv et al. (2019) combined the HCR signal amplification technique with AuNPs and electrochemical active signal molecule $[Ru(NH_3)5L]^{2+}$ in the biosensor design, where L for 3-(2-phenanthren-9-ylvinyl)-pyridine which intercalates dsDNA and decreases background noise and interferences in target detection. In their design, the AuNPs were modified with two different ssDNA sequences. One is the reporter DNA that is complementary to one end of target DNA, and another is initiator DNA for initiating HCR. The target DNA bridged AuNPs and the CPs on the electrode surface, forming a sandwich structure. Then, multiple numbers of signal indicators, $[Ru(NH_3)5L]^{2+}$ intercalated with the dsDNA replicated by HCR. This combined triple amplification strategy significantly enhanced the DNA detection signal intensity, realizing a highly sensitive and selective sensor with a detection limit as low as 0.68 aM. Ge et al. (2014) also fabricated a microRNA biosensor using HCR amplification technology on a 3D tetrahedron functionalized gold electrode (Figure 8b). The hybridization between DNA targets and tetrahedral probes triggered the HCR process by adding two types of biotin-modified hairpin species. Then, avidin-modified HRP was attached to the HCR products to produce an amplified electrocatalytic signal. Compared with a tetrahedral supported electrochemical biosensor without HCR amplification, the detection limit of this biosensor was improved by several orders of magnitude. Likewise, L. Zhu et al. (2019) combined DNA tetrahedron nanostructure with HCR signal amplification for the ultrasensitive detection of miRNA-133a. HCR-based signal amplification has become one of the widely used sensitive and cost-effective technique for electrochemical biosensor. By repeating the units of initial product, complicated DNA superstructures can be generated in HCR. Bao et al. (2019) applied this principle and fabricated the layered-branched DNA structure on the electrode surface for interferon-gamma detection 2019. Recently, beside linear chain hybridization, many new strategies have been proposed to enhance the detection efficiency (Z. Zeng et al., 2020; J. Zhang, Lakshmipriya, et al., 2020).

3.3 | Nanomaterial-based amplification

Nanomaterials have great potential for improving both sensitivity and selectivity of electrochemical biosensors because of their excellent biological compatibility, high surface area, chemical stability, excellent catalytic activity, resistance to electrode fouling, and conductivity. Therefore, the incorporation of nanoscale materials, such as carbon nanomaterials, magnetic microbeads, metal nanoparticles (NPs), and QDs, with electrochemical biosensors, has greatly improved the detection sensitivity and selectivity of sensing devices (F. Liu et al., 2020; Ozkan-Ariksoysal et al., 2017; B. Yang, Zhang, et al., 2019; C. C. Zhang, Sun, et al., 2020). Nanomaterials have been used not only for the functionalization of the electrode surface to increase the immobilization capability of DNA probes,

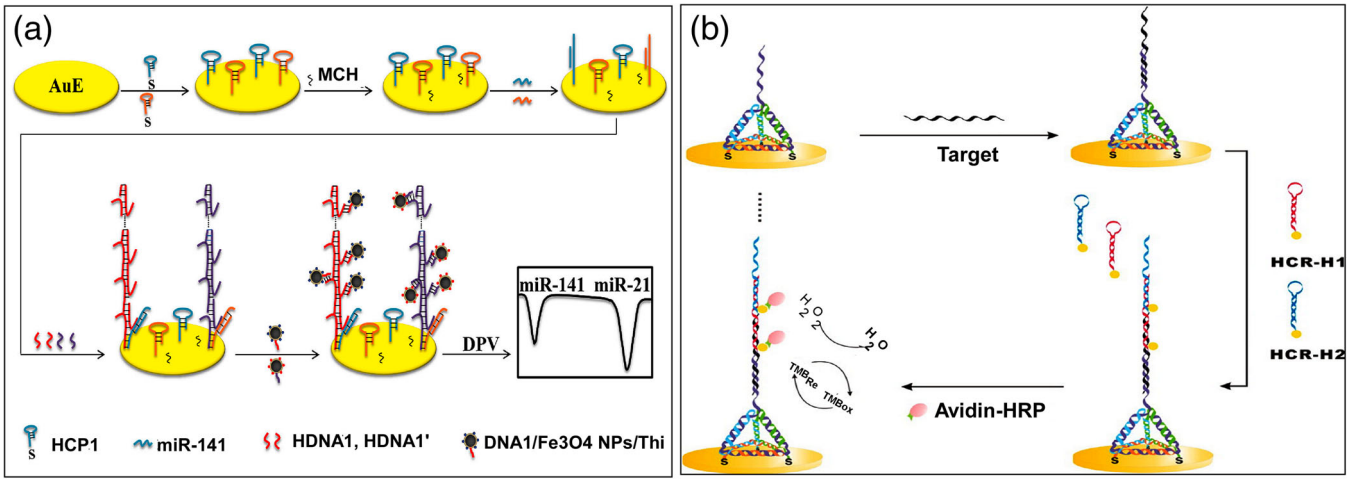

FIGURE 8 HCR-based signal amplification. (a) Thiol-modified hairpin capture probes initiating HCR in presence of target miRNA resulting in addition of series of magnetic nanoprobes for signal enhancements. (Released with permission from Yuan et al. [2017]. Copyright [2017] Elsevier B.V.). (b) Target DNA partially hybridized on tetrahedral probes triggered HCR, which bound with avidin-modified HRP producing amplified signal. (Released with permission from Ge et al. [2014], Copyright [2014] The Royal Society of Chemistry)

as described in the previous part, but also as signal amplifiers to improve the sensitivity of the electrochemical biosensors as they can easily conjugate with biomolecules (Cajigas & Orozco, 2020). Nanomaterials can assist in signal amplification in several ways; based on their role, it can be mainly classified into three main categories: nanoparticles as nanolabel, that is, redox active species, as nanocarrier to load the reporter molecules, and as nanocatalyst to facilitate the generation of electroactive species.

3.3.1 | Metal NP as nano-label

Since the nanomaterials can undergo electrochemical oxidation and reduction, they can be directly used as signal labels for target detection (Kokkinos, 2019). For example, Ye et al. (2018) used silver nanoparticle clusters (AgNCs) as labels to enhance electrochemical signals for the detection of the target invA gene of *Salmonella* (Figure 9a). In the study, the thiolated CP was covalently immobilized on the GCE and further hybridized with the target DNA and the signal DNA on AuNPs, resulting in the formation of a sandwich structure on the electrode surface. The SPs on AuNPs were cysteine-rich (C-rich), which served as templates for the formation of AgNCs. As the number of AuNPs signal DNA increased, the absolute value of the peak reduction current of AgNCs increased with increasing amounts of AgNCs upon target hybridization. The manufactured biosensor exhibited good linearity from 1 fM to 0.1 nM, and the detection limit was 0.162 fM. Ozsoz et al. (2003) used AuNPs as electroactive labels for detection of factor V Leiden mutation along with polymerase chain reaction. The pencil graphite electrode modified with mutant or wild-type target DNA of factor V Leiden was introduced into the sample containing CP modified AuNPs. The Au oxidation signal confirmed the hybridization between the target and the probe, while there was no Au oxidation signal for the noncomplementary DNA target.

Multiple nanomaterials can work synergistically to enhance electrochemical signals that a single component cannot realize. Y. Han, An, et al. (2020) proposed an ultrasensitive DNA biosensor based on a urchinlike CNT-AuNP nanocluster serving as a signal amplifier (Figure 9b). In their work, the dopamine electro-polymerization was first performed on the gold electrode to immobilize DNA CPs through the Schiff base reaction. Upon the hybridization of target nucleic acid, the dual-DNA (reporter and linker) functionalized AuNPs were introduced into the sensing system via DNA hybridization. Afterward, the end-modified single-wall CNTs with DNA (SWCNT-DNA) were attached to the surface of the AuNPs through linker-DNA hybridization to form 3D radial nanocomposites. Because of the larger contact surface area and super electronic conductivity of CNT-AuNP nanocomposites, this novel designed 3D radial nanocomposite exhibited a significant electrochemical response,

which led to ultrasensitive detection of DNA, with a detection limit of 5.2 fM and a linear range of from 0.1 pM to 10 nM, as well as a high selectivity that could discriminate single-mismatched DNA from fully matched target DNA under optimal conditions.

The chemical oxidation of metal nanoparticles like Zns, Cds, AuNPs, AgNPs, and QDs could release abundant metal cations, which can be used as a label to accommodate signal amplification (Kokkinos, 2019). For example, C. Li, Hu, Lu, Mao, et al. (2018) reported a quantum-dot (QD)-based electrochemical biosensor for accurate measurement of telomerase activity at the single-cell level. In their design, the addition of telomerase enables the addition of the telomere repeats of $(TTAGGG)_n$ to the 3′ end of the primer with attached biotins, which can assemble a large amount of streptavidin-coated QDs onto the electrode. After the acidic dissolution of QDs, the released Cd(II) can be simply quantified by anodic stripping voltammetry. The effectiveness of nanomaterials as a labeling element can be further improved by coupling with the polymer. Daneshpour et al. (2016) applied nanocomposite of polymer *N*-trimethyl chitosan (TMC) with Au and Fe_3O_4 (Fe_3O_4/TMC/Au) as an effective label for gene methylation study.

3.3.2 | Meatal NP as nano-carrier

Nanomaterials have been employed as carriers to load redox reporters to enhance the electrochemical signal amplification (Cajigas & Orozco, 2020). For example, Cui et al. designed a ultrasensitive electrochemical biosensor for polynucleotide kinase detection based on AuNP-medicated lambda exonucleases cleavage-induced signal amplification (Cui, Hu, et al., 2018; Cui, Li, et al., 2018; Cui, Lu, et al., 2018)). In this work, DNA functionalized AuNPs were immobilized on electrode surfaces through DNA hybridization. Because of AuNPs carried a significant amount of DNA strands which

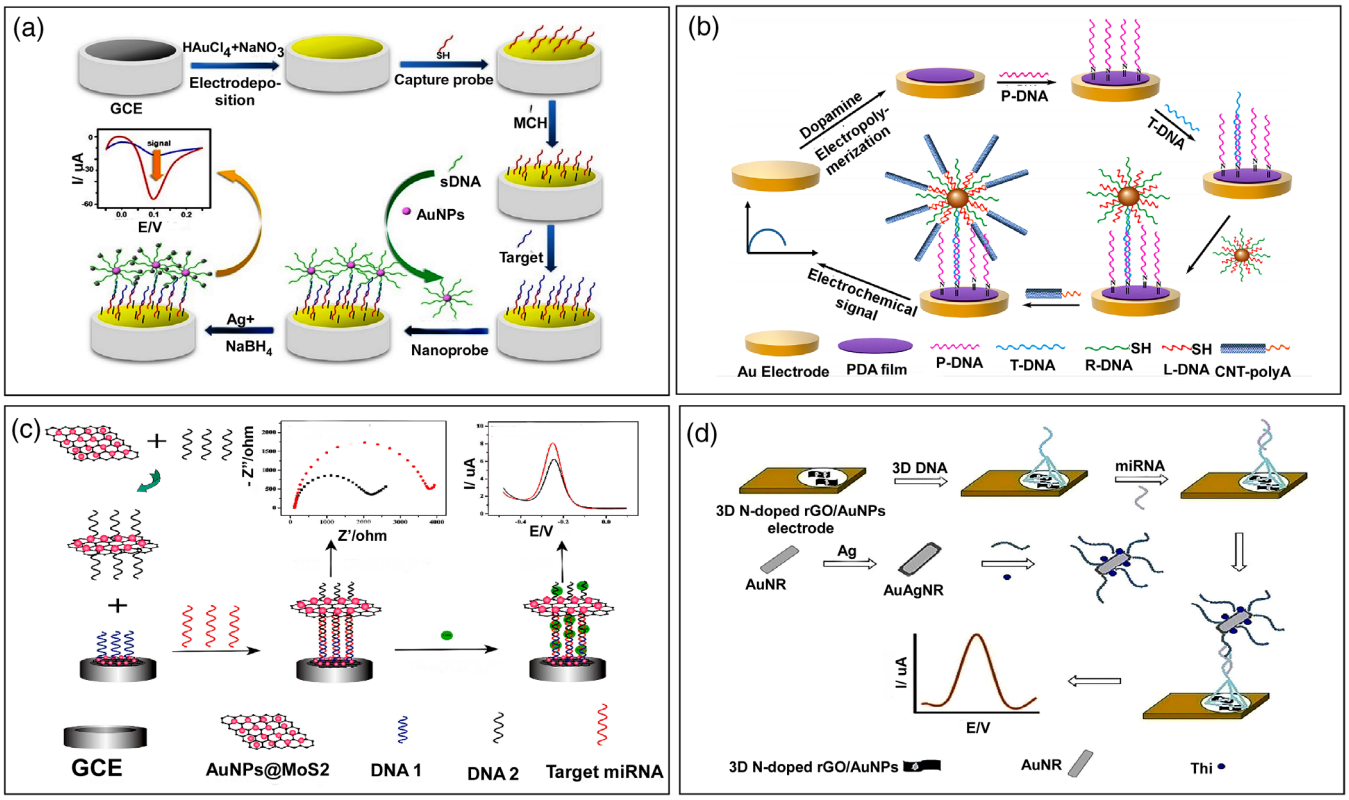

FIGURE 9 Nanomaterial-based signal amplification. (a) Silver nanoclusters integrated with DNA probes as signaling elements. (Released with permission from Ye et al. [2018]. Copyright [2018] Elsevier B.V). (b) Urchinlike carbon nanotube (CNT)-AuNP nanoclusters serving as a signal amplifier. (Released with permission from S. Han, Liu, et al. [2020]. Copyright [2020] American Chemical Society). (c) AuNPs decorated MoS_2 nanosheet as electrode modifiers and signal amplifiers. (Released with permission from Su et al. [2017]. Copyright [2017] Elsevier B.V.). (d) DNA tetrahedron immobilized on AuNP functionalized nitrogen-doped graphene oxide with Au-AgNRs and thionine composite for signal-enhancement. (Released with permission from Tian et al. [2019]. Copyright [2018] Elsevier B.V)

TABLE 2 Recent methods for signal amplification strategies

Methods	Amplification strategies	Analyte	Limit of detection	References
Enzymes-based amplification	Streptavidin modified alkaline phosphatase	Ebola virus DNA	4.7 nM	(Ilkhani & Farhad, 2018)
	Avidin-HRP	Influenza A (H7N9) virus DNA	100 fM	(Dong et al., 2015)
DNA-based amplification	Rolling circle amplification	miRNA	50 aM	(Miao et al., 2015)
	Surface-initiated enzymatic polymerization	5-Hydroxymethylcytosine (5-hmC)	9.06 fM	(Cui, Hu, et al., 2019)
	Catalytic hairpin assembly	microRNA	0.34 fM	(Ma et al., 2020)
	Hybridization chain reaction	miR-141/miR21	0.44 fM/0.46 fM	(Yuan et al., 2017)
Nanoparticles-based amplification	Silver nanoparticle	invA gene of Salmonella	0.162 fM	(Ye et al., 2018)
	Carbon nanotube (CNT)-AuNP	DNA	5.2 fM	(Y. Han, An, et al., 2020; S. Han, Liu, et al., 2020)
	Graphene	miRNA-21	0.45 fM	(Q. Xiao et al., 2019)
	AuNPs at molybdenum disulfide	miRNA-21	0.78 fM	(Su et al., 2017)
	Fe_3O_4/CeO_2 @Au magn-etite nanoparticles	microRNA-21	0.33 fM	(Y. Liu, Liu, et al., 2018; S. Liu, Yang, et al., 2018)
	Iron embedded nitrogen-rich carbon nanotubes (FeCN)	miRNA	8.53×10^{-16} M	(Cui, Wang, et al., 2019)

later bound with abundant $[Ru(NH3)6]^{3+}$ giving a higher electrochemical signal. In another work, Xiao et al. developed a biosensor for miRNA-21 detection, based on carboxylate-reduced graphene oxide (COOH-rGO)-based signal amplification and DSN-assisted target recycling (Q. Xiao et al., 2019). In this study, capture DNA (cDNA) was first self-assembled on the gold electrode's surface and hybridized with the target miRNA-21 to form cDNA/miRNA-21 hybrids. Then, the COOH-rGO was further attached to cDNA/miRNA-21 hybrids through π–π stacking interaction. This strategy increased the accumulation of electroactive methylene blue (MB) molecules, therefore, giving a significant DPV response of MB. Molybdenum disulfide (MoS_2) is another novel 2D nanomaterial used to manufacture sensing devices to improve target molecules' detection sensitivity. For instance, Shao et al. engineered an electrochemical biosensor to detect miRNA-21, which is based on AuNPs decorated MoS_2 nanosheets acting as both electrode modifiers and signal nano-amplifiers (Figure 9c; Su et al., 2017). The AuNP@MoS_2 film modified GCE was used to immobilize thiolated CP DNA1, which then hybridized with target miRNA-21. SP DNA2 was then conjugated on the AuNPs@MoS_2 nanosheet surface to amplify signals. The high loading capacity of the AuNPs@MoS_2 nanosheet for probe DNA2 could result in higher sensitivity as more indicator $[Fe(CN)_6]^{3-/4-}$ and $[Ru(NH_3)_6]^{3+}$ could be intercalated, thus causing an amplified response. The biosensor exhibited an excellent linear range from 10 fM to 1 nM, with a limit of detection of 0.78 and 0.45 fM for DPV and EIS based detection, respectively.

3.3.3 | Meatal NP as nano-catalyst

Although natural enzymes are reported to improve the sensitivity of biosensors successfully, several problems, such as poor stability, time-consuming preparation, and storage difficulty, have limited their applications (Bezinge et al., 2020). Various nonenzymatic nanomaterials have emerged as alternatives to conquer these limitations. Tian et al., (2019) designed a biosensor for highly sensitive detection of miRNA-155, which was prepared by immobilizing DNA tetrahedral supported CPs on 3D nitrogen-doped reduced graphene oxide/AuNPs electrode surface. In the presence of target miRNA-155, the gold and silver nanorod/thionine/complementary DNA nanocomposite was hybridized with targets and used as signal amplification, catalyzing the reduction of thionine as an electron mediator. With this method, the detection limit of 1×10^{-12} M was achieved (Figure 9d). Similarly, Fe_3O_4 nanoparticles are reported to have peroxidase-like properties catalyzing the reduction

of small dye molecules, such as thionine and methylene blue for signal amplification on biosensing (T. Zheng et al., 2014). Moreover, Liu et al. have reported biosensors based on target recycling, and AuNPs decorated Fe_3O_4/CeO_2 magnetite nanoparticles (MNPs) as catalysts for miRNA detection (Y. Liu, Liu, et al., 2018; S. Liu, Yang, et al., 2018). To make the electrochemical biosensor without using of unstable substrate like H_2O_2, iron embedded nitrogen-rich carbon nanotubes (FeCN) were introduced on the electrode surface by Cui, Wang, et al. (2019). The CP was immobilized on a glassy carbon electrode, which was first modified with FeCN and AuNPs. The target miRNA hybridized with the CP, then the poly A polymerase elongated the 3′OH terminal forming polyA extension, which further hybridized with T-rich probes generating dsDNA. The thionine intercalated to the dsDNA grooves was catalyzed by metal catalyst FeCN to give an electrochemical signal. With the continuous development of numerous cost-effective and convenient signal amplification strategies, nanomaterials have made great contribution on improvement of analytical performance of electrochemical DNA biosensors. Table 2 summarizes the strategies of nanomaterials for amplifying electrochemical signals.

4 | CONCLUSIONS AND PROSPECTIVE

Over the past several decades, considerable progress has been made in the development of DNA-based electrochemical biosensors, which offer significant advantages compared to other types of sensing approaches, such as rapid response, high sensitivity, selectivity, and reproducibility. This review highlighted the different probe immobilization methods and signal amplification strategies to ensure target molecules' efficient detection. All these advances contributed to the active cooperation of functional nanomaterials and the rapid development of bionanotechnology. For example, the synergistic properties of nanomaterials (nanoparticles, 2D materials, etc.) not only increased the number of immobilized probes, enhanced the electronic conductivity on electrode surface, but also converted the subtle molecular hybridization events into the amplified electrochemical signal. Recently, the employment of DNA tetrahedron as templates to immobilize DNA probes has attracted great research attention because it dramatically enhanced the accessibility of target molecules with the capability to precisely control the density of DNA probes and maintain the upright confirmation of DNA probes by varying the size of DNA tetrahedron on the electrode surface. Later, S. Han et al. (2019) introduced more diverse DNA origami into electrochemical biosensors, which offer an even larger surface area for the rational arrangement of DNA probes by merely extending the predesigned DNA staple strands. In addition, DNA origami nanostructures have shown unprecedented ability to organize and manipulate functional nanomaterials for biomedical, optical, and electronic applications. Combining DNA nanostructures and functional nanomaterials will provide new opportunities to enhance the electrochemical biosensor's detection efficiency by improving both probe immobilization and signal amplification strategies.

Despite the enormous efforts toward developing ultrasensitive electrochemical biosensors, biomarkers' analysis in real biological samples is still a challenge, such as blood, urine, and saliva. Due to the complexity of real samples and low level of biomarkers, pretreatment procedures are generally needed, including the extraction, purification, and amplification of the target molecules, which increased the time course, required sophisticated instrument, and expertise operation. Another challenge is the transition of the designed biosensor from bench to bed in an easy-to-use device. The recent advances of microfluidic technology show a great potential to integrate the sample pretreatment, hybridization, and signal readout into a lab-on-chip device. The realization of such sensitive and portable devices requires multidisciplinary collaboration from biochemistry, material science, electronics to nanofabrication. The great advances in these fields and their close collaboration will speed the development of portable electrochemical biosensors, especially in clinical and early disease diagnosis applications.

CONFLICT OF INTEREST

The authors have declared no conflict of interest for this article.

AUTHOR CONTRIBUTIONS

Krishna Thapa: writing-original draft; writing-review and editing. **Wenyan Liu**: Conceptualization; writing-original draft; writing-review and editing. **Risheng Wang**: Conceptualization; writing-review and editing; supervision.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID

Wenyan Liu https://orcid.org/0000-0002-7233-7601
Risheng Wang https://orcid.org/0000-0001-6539-1565

RELATED WIREs ARTICLES

Toward biosensors for the detection of circulating microRNA as a cancer biomarker: an overview of the challenges and successes

Application of nanotechnology in biosensors for enhancing pathogen detection

REFERENCES

Ahour, F., & Shamsi, A. (2017). Electrochemical label-free and sensitive nanobiosensing of DNA hybridization by graphene oxide modified pencil graphite electrode. *Analytical Biochemistry*, *532*, 64–71. https://doi.org/10.1016/j.ab.2017.06.004

Aydemir, N., Malmström, J., & Travas-Sejdic, J. (2016). Conducting polymer based electrochemical biosensors. *Physical Chemistry Chemical Physics*, *18*(12), 8264–8277. https://doi.org/10.1039/c5cp06830d

Bao, T., Wen, M., Wen, W., Zhang, X., & Wang, S. (2019). Ultrasensitive electrochemical biosensor of interferon-gamma based on gold nanoclusters-graphene@zeolitic imidazolate framework-8 and layered-branched hybridization chain reaction. *Sensors and Actuators B: Chemical*, *296*, 296. https://doi.org/10.1016/j.snb.2019.05.083

Bezinge, L., Suea-Ngam, A., de Mello, A. J., & Shih, C. J. (2020). Nanomaterials for molecular signal amplification in electrochemical nucleic acid biosensing: Recent advances and future prospects for point-of-care diagnostics. *Molecular Systems Design & Engineering*, *5*(1), 49–66. https://doi.org/10.1039/c9me00135b

Bi, S., Yue, S., & Zhang, S. (2017). Hybridization chain reaction: A versatile molecular tool for biosensing, bioimaging, and biomedicine. *Chemical Society Reviews*, *46*(14), 4281–4298. https://doi.org/10.1039/c7cs00055c

Bo, Y., Wang, W., Qi, J., & Huang, S. (2011). A DNA biosensor based on graphene paste electrode modified with Prussian blue and chitosan. *Analyst*, *136*(9), 1946–1951. https://doi.org/10.1039/C1AN15084G

Cai, H., Cao, X., Jiang, Y., He, P., & Fang, Y. (2003). Carbon nanotube-enhanced electrochemical DNA biosensor for DNA hybridization detection. *Analytical and Bioanalytical Chemistry*, *375*(2), 287–293. https://doi.org/10.1007/s00216-002-1652-9

Cajigas, S., & Orozco, J. (2020). Nanobioconjugates for signal amplification in electrochemical biosensing. *Molecules*, *25*(15), 3542. https://doi.org/10.3390/molecules25153542

Chao, J., Zhu, D., Zhang, Y., Wang, L., & Fan, C. (2016). DNA nanotechnology-enabled biosensors. *Biosensors and Bioelectronics*, *76*, 68–79. https://doi.org/10.1016/j.bios.2015.07.007

Chen, Y. X., Huang, K. J., & Niu, K. X. (2018). Recent advances in signal amplification strategy based on oligonucleotide and nanomaterials for microRNA detection-a review. *Biosensors and Bioelectronics*, *99*, 612–624. https://doi.org/10.1016/j.bios.2017.08.036

Chen, Z., Xie, Y., Huang, W., Qin, C., Yu, A., & Lai, G. (2019). Exonuclease-assisted target recycling for ultrasensitive electrochemical detection of microRNA at vertically aligned carbon nanotubes. *Nanoscale*, *11*(23), 11262–11269. https://doi.org/10.1039/c9nr02543j

Cho, I. H., Kim, D. H., & Park, S. (2020). Electrochemical biosensors: Perspective on functional nanomaterials for on-site analysis. *Biomaterials Research*, *24*, 6. https://doi.org/10.1186/s40824-019-0181-y

Cho, I. H., Lee, J., Kim, J., Kang, M. S., Paik, J. K., Ku, S., Cho, H. M., Irudayaraj, J., & Kim, D. H. (2018). Current technologies of electrochemical immunosensors: Perspective on signal amplification. *Sensors*, *18*(207), 1–18. https://doi.org/10.3390/s18010207

Chowdhury, A. D., Takemura, K., Li, T. C., Suzuki, T., & Park, E. Y. (2019). Electrical pulse-induced electrochemical biosensor for hepatitis E virus detection. *Nature Communications*, *10*(1), 3737. https://doi.org/10.1038/s41467-019-11644-5

Condrat, C. E., Thompson, D. C., Barbu, M. G., Bugnar, O. L., Boboc, A., Cretoiu, D., Suciu, N., Cretoiu, S. M., & Voinea, S. C. (2020). miRNAs as biomarkers in disease: Latest findings regarding their role in diagnosis and prognosis. *Cell*, *9*(276), 1–32. https://doi.org/10.3390/cells9020276

Costa, M. P., Andrade, C. A. S., Montenegro, R. A., Melo, F. L., & Oliveira, M. D. L. (2014). Self-assembled monolayers of mercaptobenzoic acid and magnetite nanoparticles as an efficient support for development of tuberculosis genosensor. *Journal of Colloid and Interface Science*, *433*, 141–148. https://doi.org/10.1016/j.jcis.2014.07.014

Cui, L., Hu, J., Wang, M., Diao, X. K., Li, C. C., & Zhang, C. Y. (2018). Mimic peroxidase- and Bi2S3 nanorod-based photoelectrochemical biosensor for signal-on detection of polynucleotide kinase. *Analytical Chemistry*, *90*(19), 11478–11485. https://doi.org/10.1021/acs.analchem.8b02673

Cui, L., Hu, J., Wang, M., Li, C. C., & Zhang, C. Y. (2019). Label-free and immobilization-free electrochemical magnetobiosensor for sensitive detection of 5-hydroxymethylcytosine in genomic DNA. *Analytical Chemistry*, *91*(2), 1232–1236. https://doi.org/10.1021/acs.analchem.8b04663

Cui, L., Li, Y., Lu, M., Tang, B., & Zhang, C. Y. (2018). An ultrasensitive electrochemical biosensor for polynucleotide kinase assay based on gold nanoparticle-mediated lambda exonuclease cleavage-induced signal amplification. *Biosensors and Bioelectronics*, *99*, 1–7. https://doi.org/10.1016/j.bios.2017.07.028

Cui, L., Lu, M., Li, Y., Tang, B., & Zhang, C. Y. (2018). A reusable ratiometric electrochemical biosensor on the basis of the binding of methylene blue to DNA with alternating AT base sequence for sensitive detection of adenosine. *Biosensors and Bioelectronics*, *102*, 87–93. https://doi.org/10.1016/j.bios.2017.11.025

Cui, L., Shen, J., Ai, S., Wang, X., & Zhang, C. Y. (2020). In-situ synthesis of covalent organic polymer thin film integrates with palladium nanoparticles for the construction of a cathodic photoelectrochemical cytosensor. *Biosensors and Bioelectronics*, *168*, 112545. https://doi.org/10.1016/j.bios.2020.112545

Cui, L., Wang, M., Sun, B., Ai, S., Wang, S., & Zhang, C. Y. (2019). Substrate-free and label-free electrocatalysis-assisted biosensor for sensitive detection of microRNA in lung cancer cells. *Chemical Communications*, *55*(8), 1172–1175. https://doi.org/10.1039/c8cc09688k

Daneshpour, M., Moradi, L. S., Izadi, P., & Omidfar, K. (2016). Femtomolar level detection of RASSF1A tumor suppressor gene methylation by electrochemical nano-genosensor based on Fe3O4/TMC/Au nanocomposite and PT-modified electrode. *Biosensors and Bioelectronics*, *77*, 1095–1103. https://doi.org/10.1016/j.bios.2015.11.007

Dong, S., Zhao, R., Zhu, J., Lu, X., Li, Y., Qiu, S., Jia, L., Jiao, X., Song, S., Fan, C., Hao, R. Z., & Song, H. B. (2015). Electrochemical DNA biosensor based on a tetrahedral nanostructure probe for the detection of avian influenza a (H7N9) virus. *ACS Applied Materials & Interfaces*, *7*(16), 8834–8842. https://doi.org/10.1021/acsami.5b01438

El Aamri, M., Yammouri, G., Mohammadi, H., Amine, A., & Korri-Youssoufi, H. (2020). Electrochemical biosensors for detection of MicroRNA as a cancer biomarker: Pros and cons. *Biosensors*, *10*(0186), 1–46. https://doi.org/10.3390/bios10110186

Fan, Z., Yao, B., Ding, Y., Zhao, J., Xie, M., & Zhang, K. (2021). Entropy-driven amplified electrochemiluminescence biosensor for RdRp gene of SARS-CoV-2 detection with self-assembled DNA tetrahedron scaffolds. *Biosensors and Bioelectronics*, *178*, 113015. https://doi.org/10.1016/j.bios.2021.113015

Fu, P., Xing, S., Xu, M., Zhao, Y., & Zhao, C. (2020). Peptide nucleic acid-based electrochemical biosensor for simultaneous detection of multiple microRNAs from cancer cells with catalytic hairpin assembly amplification. *Sensors and Actuators B: Chemical*, *305*, 305. https://doi.org/10.1016/j.snb.2019.127545

Ge, Z., Lin, M., Wang, P., Pei, H., Yan, J., Shi, J., Huang, Q., He, D., Fan, C., & Zuo, X. (2014). Hybridization chain reaction amplification of microRNA detection with a tetrahedral DNA nanostructure-based electrochemical biosensor. *Analytical Chemistry*, *86*(4), 2124–2130. https://doi.org/10.1021/ac4037262

Gerbreders, V., Krasovska, M., Mihailova, I., Ogurcovs, A., Sledevskis, E., Gerbreders, A., Tamanis, E., Kokina, I., & Plaksenkova, I. (2019). ZnO nanostructure-based electrochemical biosensor for trichinella DNA detection. *Sensing and Bio-Sensing Research*, *23*, 23. https://doi.org/10.1016/j.sbsr.2019.100276

Gong, Q., Han, H., Yang, H., Zhang, M., Sun, X., Liang, Y., Liu, Z., Zhang, W., & Qiao, J. (2019). Sensitive electrochemical DNA sensor for the detection of HIV based on a polyaniline/graphene nanocomposite. *Journal of Materiomics*, *5*(2), 313–319. https://doi.org/10.1016/j.jmat.2019.03.004

Guo, J., Yuan, C., Yan, Q., Duan, Q., Li, X., & Yi, G. (2018). An electrochemical biosensor for microRNA-196a detection based on cyclic enzymatic signal amplification and template-free DNA extension reaction with the adsorption of methylene blue. *Biosensors and Bioelectronics*, *105*, 103–108. https://doi.org/10.1016/j.bios.2018.01.036

Guo, Q., Yu, Y., Zhang, H., Cai, C., & Shen, Q. (2020). Electrochemical sensing of exosomal MicroRNA based on hybridization chain reaction signal amplification with reduced false-positive signals. *Analytical Chemistry*, *92*(7), 5302–5310. https://doi.org/10.1021/acs.analchem.9b05849

Hai, X., Li, Y., Zhu, C., Song, W., Cao, J., & Bi, S. (2020). DNA-based label-free electrochemical biosensors: From principles to applications. *Trends in Analytical Chemistry*, *133*, 116098. https://doi.org/10.1016/j.trac.2020.116098

Han, S., Liu, W., Yang, S., & Wang, R. (2019). Facile and label-free electrochemical biosensors for MicroRNA detection based on DNA origami nanostructures. *ACS Omega*, *4*(6), 11025–11031. https://doi.org/10.1021/acsomega.9b01166

Han, S., Liu, W., Zheng, M., & Wang, R. (2020). Label-free and ultrasensitive electrochemical DNA biosensor based on urchinlike carbon nanotube-gold nanoparticle nanoclusters. *Analytical Chemistry*, *92*(7), 4780–4787. https://doi.org/10.1021/acs.analchem.9b03520

Han, Y., An, F., Liu, J., Kong, J., & Zhang, X. (2020). Highly sensitive determination of DNA via a new type of electrochemical zirconium signaling probe. *New Journal of Chemistry*, *44*(47), 20770–20775. https://doi.org/10.1039/d0nj04405a

Hao, Y., Yan, P., Zhang, H., Zhang, X., Zhang, M., Shen, H., & Xiang, H. (2017). Ultrasensitive amperometric determination of PSA based on a signal amplification strategy using nanoflowers composed of single-strand DNA modified fullerene and methylene blue, and an improved surface-initiated enzymatic polymerization. *Microchimica Acta*, *184*(11), 4341–4349. https://doi.org/10.1007/s00604-017-2476-9

Herne, T. M., & Tarlov, M. J. (1997). Characterization of DNA probes immobilized on gold surfaces. *Journal of the American Chemical Society*, *119*(38), 8916–8920.

Hu, Y., Xu, X., Liu, Q., Wang, L., Lin, Z., & Chen, G. (2014). Ultrasensitive electrochemical biosensor for detection of DNA from Bacillus subtilis by coupling target-induced strand displacement and nicking endonuclease signal amplification. *Analytical Chemistry*, *86*(17), 8785–8790. https://doi.org/10.1021/ac502008k

Huang, J. Y., Zhao, L., Lei, W., Wen, W., Wang, Y. J., Bao, T., Xiong, H. Y., Zhang, X. H., & Wang, S. F. (2018). A high-sensitivity electrochemical aptasensor of carcinoembryonic antigen based on graphene quantum dots-ionic liquid-nafion nanomatrix and DNAzyme-assisted signal amplification strategy. *Biosensors and Bioelectronics*, *99*, 28–33. https://doi.org/10.1016/j.bios.2017.07.036

Huang, Y. L., Mo, S., Gao, Z. F., Chen, J. R., Lei, J. L., Luo, H. Q., & Li, N. B. (2017). Amperometric biosensor for microRNA based on the use of tetrahedral DNA nanostructure probes and guanine nanowire amplification. *Microchimica Acta*, *184*(8), 2597–2604. https://doi.org/10.1007/s00604-017-2246-8

Ilkhani, H., & Farhad, S. (2018). A novel electrochemical DNA biosensor for Ebola virus detection. *Analytical Biochemistry*, *557*, 151–155. https://doi.org/10.1016/j.ab.2018.06.010

Jahanbani, S., & Benvidi, A. (2016). A novel electrochemical DNA biosensor based on a modified magnetic bar carbon paste electrode with Fe3O4NPs-reduced graphene oxide/PANHS nanocomposite. *Materials Science and Engineering C*, *68*, 1–8. https://doi.org/10.1016/j.msec.2016.05.056

Jain, A., & Cheng, K. (2017). The principles and applications of avidin-based nanoparticles in drug delivery and diagnosis. *Journal of Controlled Release*, *245*, 27–40. https://doi.org/10.1016/j.jconrel.2016.11.016

Ji, Y., Zhang, L., Zhu, L., Lei, J., Wu, J., & Ju, H. (2017). Binding-induced DNA walker for signal amplification in highly selective electrochemical detection of protein. *Biosensors and Bioelectronics*, *96*, 201–205. https://doi.org/10.1016/j.bios.2017.05.008

Jia, S., Wang, J., Xie, M., Sun, J., Liu, H., Zhang, Y., Chao, J., Li, J., Wang, L., Lin, J., Gothelf, K. V., & Fan, C. (2019). Programming DNA origami patterning with non-canonical DNA-based metallization reactions. *Nature Communications*, *10*(1), 5597. https://doi.org/10.1038/s41467-019-13507-5

Ke, Y., Lindsay, S., Chang, Y., Liu, Y., & Yan, H. (2008). Self-assembled water-soluble nucleic acid probe tiles for label-free RNA hybridization assays. *Science*, *319*(5860), 180–183. https://doi.org/10.1126/science.1150082

Kjällman, T. H., Peng, H., Soeller, C., & Travas-Sejdic, J. (2008). Effect of probe density and hybridization temperature on the response of an electrochemical hairpin-DNA sensor. *Analytical Chemistry*, *80*, 9460–9466.

Kokkinos, C. (2019). Electrochemical DNA biosensors based on labeling with nanoparticles. *Nanomaterials*, *9*(1361), 1–20. https://doi.org/10.3390/nano9101361

Lang, M., Luo, D., Yang, G., Mei, Q., Feng, G., Yang, Y., Liu, Z., Chen, Q., & Wu, L. (2020). An ultrasensitive electrochemical sensing platform for the detection of cTnI based on aptamer recognition and signal amplification assisted by TdT. *RSC Advances*, *10*(60), 36396–36403. https://doi.org/10.1039/d0ra05171c

Li, C., Hu, X., Lu, J., Mao, X., Xiang, Y., Shu, Y., & Li, G. (2018). Design of DNA nanostructure-based interfacial probes for the electrochemical detection of nucleic acids directly in whole blood. *Chemical Science*, *9*(4), 979–984. https://doi.org/10.1039/c7sc04663d

Li, C. C., Hu, J., Lu, M., & Zhang, C. Y. (2018). Quantum dot-based electrochemical biosensor for stripping voltammetric detection of telomerase at the single-cell level. *Biosensors and Bioelectronics*, *122*, 51–57. https://doi.org/10.1016/j.bios.2018.09.049

Li, X., Li, X., Li, D., Zhao, M., Wu, H., Shen, B., Liu, P., & Ding, S. (2020). Electrochemical biosensor for ultrasensitive exosomal miRNA analysis by cascade primer exchange reaction and MOF@Pt@MOF nanozyme. *Biosensors and Bioelectronics*, *168*, 112554. https://doi.org/10.1016/j.bios.2020.112554

Li, Z., Mohamed, M. A., Vinu Mohan, A. M., Zhu, Z., Sharma, V., Mishra, G. K., & Mishra, R. K. (2019). Application of electrochemical aptasensors toward clinical diagnostics, food, and environmental monitoring: Review. *Sensors*, *19*(5435), 1–22. https://doi.org/10.3390/s19245435

Liang, M., Pan, M., Hu, J., Wang, F., & Liu, X. (2018). Electrochemical biosensor for MicroRNA detection based on cascade hybridization chain reaction. *ChemElectroChem*, *5*(10), 1380–1386. https://doi.org/10.1002/celc.201800255

Lichtenberg, J. Y., Ling, Y., & Kim, S. (2019). Non-specific adsorption reduction methods in biosensing. *Sensors*, *19*(2488), 1–17. https://doi.org/10.3390/s19112488

Lin, J., Gopinath, S. C. B., Lakshmipriya, T., Chen, Y., Yuan, W. R., & Yang, M. (2019). Target DNA detection of human papilloma virus-16 E7 gene by capture-target-reporter sandwich on interdigitated electrode sensor. *International Journal of Biological Macromolecules*, *141*, 564–569. https://doi.org/10.1016/j.ijbiomac.2019.09.012

Lin, M., Wang, J., Zhou, G., Wang, J., Wu, N., Lu, J., Gao, J., Chen, X., Shi, J., Zuo, X., & Fan, C. (2015). Programmable engineering of a biosensing interface with tetrahedral DNA nanostructures for ultrasensitive DNA detection. *Angewandte Chemie International Edition*, *54*(7), 2151–2155. https://doi.org/10.1002/anie.201410720

Liu, A., Chen, X., Wang, K., Wei, N., Sun, Z., Lin, X., Chen, Y., & Du, M. (2011a). Electrochemical DNA biosensor based on aldehyde-agarose hydrogel modified glassy carbon electrode for detection of PML/RARa fusion gene. *Sensors and Actuators B: Chemical*, *160*(1), 1458–1463. https://doi.org/10.1016/j.snb.2011.10.013

Liu, W., Zhong, H., Wang, R., & Seeman, N. C. (2011b). Crystalline two-dimensional DNA origami arrays. *Angew Chem Int Ed Engl*, *50*(1), 264–267. https://doi.org/10.1002/anie.201005911

Liu, F., Li, K., Zhang, Y., Ding, J., Wen, T., Pei, X., Yan, Y., Ji, W., Liu, J., Zhang, X., & Li, L. (2020). An electrochemical DNA biosensor based on nitrogen-doped graphene nanosheets decorated with gold nanoparticles for genetically modified maize detection. *Mikrochimca Acta*, *187*(10), 574. https://doi.org/10.1007/s00604-020-04511-4

Liu, G., Wan, Y., Gau, V., Zhang, J., Wang, L., Song, S., & Fan, C. (2008). An enzyme-based E-DNA sensor for sequence-specific detection of femtomolar DNA targets. *Journal of the American Chemical Society*, *130*(21), 6820–6825. https://doi.org/10.1021/ja800554t

Liu, S., Yang, Z., Chang, Y., Chai, Y., & Yuan, R. (2018). An enzyme-free electrochemical biosensor combining target recycling with Fe3O4/CeO2@Au nanocatalysts for microRNA-21 detection. *Biosensors and Bioelectronics*, *119*, 170–175. https://doi.org/10.1016/j.bios.2018.08.006

Liu, W., Li, L., Yang, S., Gao, J., & Wang, R. (2017). Self-assembly of heterogeneously shaped nanoparticles into plasmonic metamolecules on DNA origami. *Chemistry*, *23*(57), 14177–14181. https://doi.org/10.1002/chem.201703927

Liu, W., Tagawa, M., Xin, H. L., Wang, T., Emamy, H., Li, H., Yager, K. G., Starr, F. W., Tkachenko, A. V., & Gang, O. (2016). Diamond family of nanoparticle superlattices. *Science*, *351*(6273), 582–586. https://doi.org/10.1126/science.aad2080

Liu, Y., Liu, Y., Qiao, L., Liu, Y., & Liu, B. (2018). Advances in signal amplification strategies for electrochemical biosensing. *Current Opinion in Electrochemistry*, *12*, 5–12. https://doi.org/10.1016/j.coelec.2018.05.001

Liu, Y. H., Li, H. N., Chen, W., Liu, A. L., Lin, X. H., & Chen, Y. Z. (2013). Bovine serum albumin-based probe carrier platform for electrochemical DNA biosensing. *Analytical Chemistry*, *85*(1), 273–277. https://doi.org/10.1021/ac303397f

Lv, M. M., Fan, S. F., Wang, Q. L., Lv, Q. Y., Song, X., & Cui, H. F. (2019). An enzyme-free electrochemical sandwich DNA assay based on the use of hybridization chain reaction and gold nanoparticles: Application to the determination of the DNA of helicobacter pylori. *Mikrochimica Acta*, *187*(1), 73. https://doi.org/10.1007/s00604-019-3999-z

Ma, X., Qian, K., Ejeromedoghene, O., Kandawa-Schulz, M., & Wang, Y. (2020). Electrochemical detection of microRNA based on SA-PPy/AuNPs nanocomposite with the signal amplification through catalytic hairpin assembly reaction and the spontaneous catalytic reaction of Fe3+/Cu2+. *Electrochimica Acta*, *362*, 362. https://doi.org/10.1016/j.electacta.2020.137168

Maduraiveeran, G., Sasidharan, M., & Ganesan, V. (2018). Electrochemical sensor and biosensor platforms based on advanced nanomaterials for biological and biomedical applications. *Biosensors and Bioelectronics*, *103*, 113–129. https://doi.org/10.1016/j.bios.2017.12.031

Mahmudunnabi, R. G., Farhana, F. Z., Kashaninejad, N., Firoz, S. H., Shim, Y. B., & Shiddiky, M. J. A. (2020). Nanozyme-based electrochemical biosensors for disease biomarker detection. *Analyst*, *145*(13), 4398–4420. https://doi.org/10.1039/d0an00558d

Miao, P., Wang, B., Meng, F., Yin, J., & Tang, Y. (2015). Ultrasensitive detection of microRNA through rolling circle amplification on a DNA tetrahedron decorated electrode. *Bioconjugate Chemistry*, *26*(3), 602–607. https://doi.org/10.1021/acs.bioconjchem.5b00064

Miao, P., Zhang, T., Xu, J., & Tang, Y. (2018). Electrochemical detection of miRNA combining T7 exonuclease-assisted cascade signal amplification and DNA-templated copper nanoparticles. *Analytical Chemistry*, *90*(18), 11154–11160. https://doi.org/10.1021/acs.analchem.8b03425

Mohammed, A. M., Rahim, R. A., Ibraheem, I. J., Loong, F. K., Hisham, H., Hashim, U., & Al-Douri, Y. (2014). Application of gold nanoparticles for electrochemical DNA biosensor. *Journal of Nanomaterials*, *2014*, 1–7. https://doi.org/10.1155/2014/683460

Mohsen, M. G., & Kool, E. T. (2016). The discovery of rolling circle amplification and rolling circle transcription. *Accounts of Chemical Research*, *49*(11), 2540–2550. https://doi.org/10.1021/acs.accounts.6b00417

Nemiroski, A., Christodouleas, D. C., Hennek, J. W., Kumar, A. A., Maxwell, E. J., Fernandez-Abedul, M. T., & Whitesides, G. M. (2014). Universal mobile electrochemical detector designed for use in resource-limited applications. *Proceedings of the National Academy of Sciences*, *111*(33), 11984–11989. https://doi.org/10.1073/pnas.1405679111

Nimse, S. B., Song, K., Sonawane, M. D., Sayyed, D. R., & Kim, T. (2014). Immobilization techniques for microarray: Challenges and applications. *Sensors*, *14*(12), 22208–22229. https://doi.org/10.3390/s141222208

Niu, X., Zheng, W., Yin, C., Weng, W., Li, G., Sun, W., & Men, Y. (2017). Electrochemical DNA biosensor based on gold nanoparticles and partially reduced graphene oxide modified electrode for the detection of Listeria monocytogenes hly gene sequence. *Journal of Electroanalytical Chemistry*, *806*, 116–122. https://doi.org/10.1016/j.jelechem.2017.10.049

Ozkan-Ariksoysal, D., Kayran, Y. U., Yilmaz, F. F., Ciucu, A. A., David, I. G., David, V., Hosgor-Limoncu, M., & Ozsoz, M. (2017). DNA-wrapped multi-walled carbon nanotube modified electrochemical biosensor for the detection of Escherichia coli from real samples. *Talanta*, *166*, 27–35. https://doi.org/10.1016/j.talanta.2017.01.005

Ozsoz, M., Erdem, A., Kerman, K., Ozkan, D., Tugrul, B., Topcuoglu, N., Ekren, H., & Taylan, M. (2003). Electrochemical genosensor based on colloidal gold nanoparticles for the detection of factor V Leiden mutation using disposable pencil graphite electrodes. *Analytical Chemistry*, *75*, 2181–2187.

Pei, H., Lu, N., Wen, Y., Song, S., Liu, Y., Yan, H., & Fan, C. (2010). A DNA nanostructure-based biomolecular probe carrier platform for electrochemical biosensing. *Advanced Materials*, *22*(42), 4754–4758. https://doi.org/10.1002/adma.201002767

Pei, H., Zuo, X., Pan, D., Shi, J., Huang, Q., & Fan, C. (2013). Scaffolded biosensors with designed DNA nanostructures. *NPG Asia Materials*, *5*(6), e51–e51. https://doi.org/10.1038/am.2013.22

Rahman, M. M., Li, X. B., Lopa, N. S., Ahn, S. J., & Lee, J. J. (2015). Electrochemical DNA hybridization sensors based on conducting polymers. *Sensors*, *15*(2), 3801–3829. https://doi.org/10.3390/s150203801

Ronkainen, N. J., Brain Halsall, H., & Heineman, W. R. (2010). Electrochemical biosensors. *Chemical Society Reviews*, *39*(5), 1747–1763. https://doi.org/10.1039/b714449k

Sarkar, S., Sabhachandani, P., & Konry, T. (2017). Isothermal amplification strategies for detection in microfluidic devices. *Trends in Biotechnology*, *35*(3), 186–189. https://doi.org/10.1016/j.tibtech.2016.09.006

Seeman, N. C., & Sleiman, H. F. (2017). DNA nanotechnology. *Nature Reviews Materials*, *3*(17068). https://doi.org/10.1038/natrevmats.2017.68

Settu, K., Liu, J. T., Chen, C. J., & Tsai, J. Z. (2017). Development of carbon-graphene-based aptamer biosensor for EN2 protein detection. *Analytical Biochemistry*, *534*, 99–107. https://doi.org/10.1016/j.ab.2017.07.012

Shuai, H. L., Huang, K. J., Xing, L. L., & Chen, Y. X. (2016). Ultrasensitive electrochemical sensing platform for microRNA based on tungsten oxide-graphene composites coupling with catalyzed hairpin assembly target recycling and enzyme signal amplification. *Biosensors and Bioelectronics*, *86*, 337–345. https://doi.org/10.1016/j.bios.2016.06.057

Smith, C. L., Milea, J. S., & Nguyen, G. H. (2006). Immobilization of nucleic acids using biotin-Strept(avidin) systems. In *Topics in current chemistry*, Immobilisation of DNA on chips II, (Vol. 416, pp. 63–90). https://doi.org/10.1007/128_017

Su, S., Cao, W., Liu, W., Lu, Z., Zhu, D., Chao, J., Weng, L., Wang, L., Fan, C., & Wang, L. (2017). Dual-mode electrochemical analysis of microRNA-21 using gold nanoparticle-decorated MoS2 nanosheet. *Biosensors and Bioelectronics*, *94*, 552–559. https://doi.org/10.1016/j.bios.2017.03.040

Subramanian, H. K., Chakraborty, B., Sha, R., & Seeman, N. C. (2011). The label-free unambiguous detection and symbolic display of single nucleotide polymorphisms on DNA origami. *Nano Letters*, *11*(2), 910–913. https://doi.org/10.1021/nl104555t

Swarbrick, S., Wragg, N., Ghosh, S., & Stolzing, A. (2019). Systematic review of miRNA as biomarkers in Alzheimer's disease. *Molecular Neurobiology*, *56*(9), 6156–6167. https://doi.org/10.1007/s12035-019-1500-y

Teles, F., & Fonseca, L. (2008). Trends in DNA biosensors. *Talanta*, *77*(2), 606–623. https://doi.org/10.1016/j.talanta.2008.07.024

Tian, L., Zhang, Y., Wang, L., Geng, Q., Liu, D., Duan, L., Wang, Y., & Cui, J. (2020). Ratiometric dual signal-enhancing-based electrochemical biosensor for ultrasensitive kanamycin detection. *ACS Applied Materials and Interfaces*, *12*(47), 52713–52720. https://doi.org/10.1021/acsami.0c15898

Tian, R., Ning, W., Chen, M., Zhang, C., Li, Q., & Bai, J. (2019). High performance electrochemical biosensor based on 3D nitrogen-doped reduced graphene oxide electrode and tetrahedral DNA nanostructure. *Talanta*, *194*, 273–281. https://doi.org/10.1016/j.talanta.2018.09.110

Wang, D., Hu, L., Zhou, H., Abdel-Halim, E. S., & Zhu, J. J. (2013). Molecular beacon structure mediated rolling circle amplification for ultrasensitive electrochemical detection of microRNA based on quantum dots tagging. *Electrochemistry Communications*, *33*, 80–83. https://doi.org/10.1016/j.elecom.2013.04.030

Wang, J., Shi, A., Fang, X., Han, X., & Zhang, Y. (2014). Ultrasensitive electrochemical supersandwich DNA biosensor using a glassy carbon electrode modified with gold particle-decorated sheets of graphene oxide. *Microchimica Acta*, *181*(9–10), 935–940. https://doi.org/10.1007/s00604-014-1182-0

Wang, J. L., Lu, J., Dong, S., Zhu, N., Gyimah, E., Wang, K., Li, Y., & Zhang, Z. (2019). An ultrasensitive electrochemical biosensor for detection of microRNA-21 based on redox reaction of ascorbic acid/iodine and duplex-specific nuclease assisted target recycling. *Biosensors and Bioelectronics*, *130*, 81–87. https://doi.org/10.1016/j.bios.2019.01.031

Wang, L., Liao, X., Ding, Y., Gao, F., & Wang, Q. (2013). DNA biosensor based on a glassy carbon electrode modified with electropolymerized Eriochrome Black T. *Microchimica Acta*, *181*(1–2), 155–162. https://doi.org/10.1007/s00604-013-1085-5

Wang, Q., Gao, F., Ni, J., Liao, X., Zhang, X., & Lin, Z. (2016). Facile construction of a highly sensitive DNA biosensor by in-situ assembly of electro-active tags on hairpin-structured probe fragment. *Scientific Reports*, *6*, 22441. https://doi.org/10.1038/srep22441

Wang, Q., Zhang, B., Lin, X., & Weng, W. (2011). Hybridization biosensor based on the covalent immobilization of probe DNA on chitosan–mutiwalled carbon nanotubes nanocomposite by using glutaraldehyde as an arm linker. *Sensors and Actuators B: Chemical*, *156*(2), 599–605. https://doi.org/10.1016/j.snb.2011.02.004

Wang, R., Nuckolls, C., & Wind, S. J. (2012). Assembly of heterogeneous functional nanomaterials on DNA origami scaffolds. *Angewandte Chemie International Edition*, *51*(45), 11325–11327. https://doi.org/10.1002/anie.201206389

Wang, X., Niu, S., Wei, M., Liu, S., Liu, R., Shi, C., & Ma, C. (2019). Ultrasensitive electrochemical DNA biosensor based on a tetrahedral structure and proximity-dependent surface hybridization. *Analyst*, *145*(1), 150–156. https://doi.org/10.1039/c9an01897b

Wang, Y. H., He, L. L., Huang, K. J., Chen, Y. X., Wang, S. Y., Liu, Z. H., & Li, D. (2019). Recent advances in nanomaterial-based electrochemical and optical sensing platforms for microRNA assays. *Analyst*, *144*(9), 2849–2866. https://doi.org/10.1039/c9an00081j

White, R. J., Phares, N., Lubin, A. A., Xiao, Y., & Plaxco, K. W. (2008). Optimization of electrochemical aptamer-based sensors via optimization of probe packing density and surface chemistry. *Langmuir*, *24*, 10513–10518. https://doi.org/10.1021/la800801v

Wu, Y., Cui, S., Li, Q., Zhang, R., Song, R., Gao, Y., Chen, W., & Xing, D. (2020). Recent advances in duplex-specific nuclease-based signal amplification strategies for microRNA detection. *Biosensors and Bioelectronics*, *165*, 112449. https://doi.org/10.1016/j.bios.2020.112449

Xia, N., Zhang, Y., Wei, X., Huang, Y., & Liu, L. (2015). An electrochemical microRNAs biosensor with the signal amplification of alkaline phosphatase and electrochemical-chemical-chemical redox cycling. *Analytica Chimica Acta*, *878*, 95–101. https://doi.org/10.1016/j.aca.2015.04.018

Xiao, F., Liu, J., Guo, Q., Du, Z., Li, H., Sun, C., & Du, W. (2020). Dual-signal amplification strategy for sensitive MicroRNA detection based on rolling circle amplification and enzymatic repairing amplification. *ACS Omega*, *5*(50), 32738–32743. https://doi.org/10.1021/acsomega.0c05141

Xiao, Q., Li, J., Jin, X., Liu, Y., & Huang, S. (2019). Ultrasensitive electrochemical microRNA-21 biosensor coupling with carboxylate-reduced graphene oxide-based signal-enhancing and duplex-specific nuclease-assisted target recycling. *Sensors and Actuators B: Chemical*, *297*, 297. https://doi.org/10.1016/j.snb.2019.126740

Xiao, Y., Qu, X., Plaxco, K. W., & Heeger, A. J. (2007). Label-free electrochemical detection of DNA in blood serum via target-induced resolution of an electrode-bound DNA pseudoknot. *Journal of the American Chemical Society*, *129*, 11896–11897. https://doi.org/10.1021/ja074218y

Xu, B., Zheng, D., Qiu, W., Gao, F., Jiang, S., & Wang, Q. (2015). An ultrasensitive DNA biosensor based on covalent immobilization of probe DNA on fern leaf-like alpha-Fe2O3 and chitosan hybrid film using terephthalaldehyde as arm-linker. *Biosensors and Bioelectronics*, *72*, 175–181. https://doi.org/10.1016/j.bios.2015.05.015

Xu, C. X., Zhai, Q. G., Liu, Y. J., Huang, K. J., Lu, L., & Li, K. X. (2014). A novel electrochemical DNA biosensor construction based on layered CuS-graphene composite and au nanoparticles. *Analytical and Bioanalytical Chemistry*, *406*(27), 6943–6951. https://doi.org/10.1007/s00216-014-7904-7

Xu, L., Duan, J., Chen, J., Ding, S., & Cheng, W. (2021). Recent advances in rolling circle amplification-based biosensing strategies-a review. *Analytica Chimica Acta*, *1148*, 238187. https://doi.org/10.1016/j.aca.2020.12.062

Xu, L., Liang, W., Wen, Y., Wang, L., Yang, X., Ren, S., Jia, N., Zuo, X., & Liu, G. (2018). An ultrasensitive electrochemical biosensor for the detection of mecA gene in methicillin-resistant Staphylococcus aureus. *Biosensors and Bioelectronics*, *99*, 424–430. https://doi.org/10.1016/j.bios.2017.08.014

Yang, B., Zhang, S., Fang, X., & Kong, J. (2019). Double signal amplification strategy for ultrasensitive electrochemical biosensor based on nuclease and quantum dot-DNA nanocomposites in the detection of breast cancer 1 gene mutation. *Biosensors and Bioelectronics*, *142*, 111544. https://doi.org/10.1016/j.bios.2019.111544

Yang, F., Zuo, X., Fan, C., & Zhang, X. E. (2018). Biomacromolecular nanostructures-based interfacial engineering: From precise assembly to precision biosensing. *National Science Review*, *5*(5), 740–755. https://doi.org/10.1093/nsr/nwx134

Yang, J., Gao, L., Peng, C., & Zhang, W. (2019). Construction of self-signal DNA electrochemical biosensor employing WS2 nanosheets combined with PIn6COOH. *RSC Advances*, *9*(17), 9613–9619. https://doi.org/10.1039/c8ra10266j

Yang, L., Lia, X., Yana, S., Wanga, M., Liua, P., Donga, Y., & Zhangb, C. (2015). Single-walled carbon nanotubes-carboxyl functionalized graphene oxide based electrochemical DNA biosensor for thermolabilehemolysin gene detection. *Analytical Methods*, *12*(7), 5303–5310. https://doi.org/10.1039/C5AY01062D

Yang, L., Wang, J., Lu, H., & Hui, N. (2021). Electrochemical sensor based on Prussian blue/multi-walled carbon nanotubes functionalized polypyrrole nanowire arrays for hydrogen peroxide and microRNA detection. *Mikrochimica Acta*, *188*(1), 25. https://doi.org/10.1007/s00604-020-04673-1

Ye, Y., Liu, Y., He, S., Xu, X., Cao, X., Ye, Y., & Zheng, H. (2018). Ultrasensitive electrochemical DNA sensor for virulence invA gene of salmonella using silver nanoclusters as signal probe. *Sensors and Actuators B: Chemical*, *272*, 53–59. https://doi.org/10.1016/j.snb.2018.05.133

Yesil, M., Donmez, S., & Arslan, F. (2016). Development of an electrochemical DNA biosensor for detection of specific mycobacterium tuberculosis sequence based on poly(L-glutamic acid) modified electrode. *Journal of Chemical Sciences*, *128*(11), 1823–1829. https://doi.org/10.1007/s12039-016-1159-0

You, H., Bai, L., Yuan, Y., Zhou, J., Bai, Y., & Mu, Z. (2018). An amperometric aptasensor for ultrasensitive detection of sulfadimethoxine based on exonuclease-assisted target recycling and new signal tracer for amplification. *Biosensors and Bioelectronics*, *117*, 706–712. https://doi.org/10.1016/j.bios.2018.06.011

Yuan, Y. H., Wu, Y. D., Chi, B. Z., Wen, S. H., Liang, R. P., & Qiu, J. D. (2017). Simultaneously electrochemical detection of microRNAs based on multifunctional magnetic nanoparticles probe coupling with hybridization chain reaction. *Biosensors and Bioelectronics*, *97*, 325–331. https://doi.org/10.1016/j.bios.2017.06.022

Zahid, M., Kim, B., Hussain, R., Amin, R., & Park, S. H. (2013). DNA nanotechnology: A future perspective. *Nanoscale Research Letters*, *8*(1), 1–13. https://doi.org/10.1186/1556-276X-8-119

Zeng, D., Wang, Z., Meng, Z., Wang, P., San, L., Wang, W., Aldalbahi, A., Li, L., Shen, J., & Mi, X. (2017). DNA tetrahedral nanostructure-based electrochemical miRNA biosensor for simultaneous detection of multiple miRNAs in pancreatic carcinoma. *ACS Applied Materials and Interfaces*, *9*(28), 24118–24125. https://doi.org/10.1021/acsami.7b05981

Zeng, Z., Zhou, R., Sun, R., Zhang, X., Cheng, Z., Chen, C., & Zhu, Q. (2020). Nonlinear hybridization chain reaction-based functional DNA nanostructure assembly for biosensing, bioimaging applications. *Biosensors and Bioelectronics*, *173*, 112814. https://doi.org/10.1016/j.bios.2020.112814

Zhang, C., Chen, J., Sun, R., Huang, Z., Luo, Z., Zhou, C., Wu, M., Duan, Y., & Li, Y. (2020). The recent development of hybridization chain reaction strategies in biosensors. *ACS Sensors*, *5*(10), 2977–3000. https://doi.org/10.1021/acssensors.0c01453

Zhang, F., Nangreave, J., Liu, Y., & Yan, H. (2014). Structural DNA nanotechnology: State of the art and future perspective. *Journal of the American Chemical Society*, *136*(32), 11198–11211. https://doi.org/10.1021/ja505101a

Zhang, H., Fan, M., Jiang, J., Shen, Q., Cai, C., & Shen, J. (2019). Sensitive electrochemical biosensor for MicroRNAs based on duplex-specific nuclease-assisted target recycling followed with gold nanoparticles and enzymatic signal amplification. *Analytica Chimica Acta*, *1064*, 33–39. https://doi.org/10.1016/j.aca.2019.02.060

Zhang, J., Lakshmipriya, T., & Gopinath, S. C. B. (2020). Electroanalysis on an interdigitated electrode for high-affinity cardiac troponin I biomarker detection by aptamer-gold conjugates. *ACS Omega*, *5*(40), 25899–25905. https://doi.org/10.1021/acsomega.0c03260

Zhang, J., Lao, R., Song, S., Yan, Z., & Fan, C. (2008). Design of an Oligonucleotide-Incorporated Nonfouling Surface and its Application in electrochemical DNA sensors for highly sensitive and sequence-specific detection of target DNA. *Analytical Chemistry*, *80*(23), 9029–9033. https://doi.org/10.1021/ac801424y

Zhang, L., Deng, H., Yuan, R., & Yuan, Y. (2019). Electrochemical lead(II) biosensor by using an ion-dependent split DNAzyme and a template-free DNA extension reaction for signal amplification. *Mikrochimica Acta*, *186*(11), 709. https://doi.org/10.1007/s00604-019-3857-z

Zhang, M., Hong, H., Fen-Yue, Z., Zhong, J. C., & Jian-Ping, L. (2018). Electrochemical luminescent DNA sensor based on polymerase-assisted signal amplification. *Chinese Journal of Analytical Chemistry*, *46*(2), 203–209. https://doi.org/10.1016/s1872-2040(17)61067-3

Zheng, J., Birktoft, J. J., Chen, Y., Wang, T., Sha, R., Constantinou, P. E., Ginell, S. L., Mao, C., & Seeman, N. C. (2009). From molecular to macroscopic via the rational design of a self-assembled 3D DNA crystal. *Nature*, *461*(7260), 74–77. https://doi.org/10.1038/nature08274

Zheng, T., Zhang, Q., Feng, S., Zhu, J. J., Wang, Q., & Wang, H. (2014). Robust nonenzymatic hybrid nanoelectrocatalysts for signal amplification toward ultrasensitive electrochemical cytosensing. *Journal of the American Chemical Society*, *136*(6), 2288–2291. https://doi.org/10.1021/ja500169y

Zhou, H., Duan, S., Huang, J., & He, F. (2020). An ultrasensitive electrochemical biosensor for Pseudomonas aeruginosa assay based on a rolling circle amplification-assisted multipedal DNA walker. *Chemical Communications*, *56*(46), 6273–6276. https://doi.org/10.1039/d0cc01619e

Zhou, H., Liu, J., Xu, J. J., & Chen, H. Y. (2011). Highly sensitive electrochemiluminescence detection of single-nucleotide polymorphisms based on isothermal cycle-assisted triple-stem probe with dual-nanoparticle label. *Analytical Chemistry*, *83*(21), 8320–8328. https://doi.org/10.1021/ac2022629

Zhu, D., Yan, Y., Lei, P., Shen, B., Cheng, W., Ju, H., & Ding, S. (2014). A novel electrochemical sensing strategy for rapid and ultrasensitive detection of Salmonella by rolling circle amplification and DNA-AuNPs probe. *Analytica Chimica Acta*, *846*, 44–50. https://doi.org/10.1016/j.aca.2014.07.024

Zhu, J., Feng, X., Lou, J., Li, W., Li, S., Zhu, H., Yang, L., Zhang, A., He, L., & Li, C. (2013). Accurate quantification of microRNA via single strand displacement reaction on DNA origami motif. *PLoS One*, *8*(8), e69856. https://doi.org/10.1371/journal.pone.0069856

Zhu, L., Luo, L., & Wang, Z. (2012). DNA electrochemical biosensor based on thionine-graphene nanocomposite. *Biosensors and Bioelectronics*, *35*(1), 507–511. https://doi.org/10.1016/j.bios.2012.03.026

Zhu, L., Ye, J., Wang, S., Yan, M., Zhu, Q., Huang, J., & Yang, X. (2019). Dual amplification ratiometric biosensor based on a DNA tetrahedron nanostructure and hybridization chain reaction for the ultrasensitive detection of microRNA-133a. *Chemical Communications*, *55*(77), 11551–11554. https://doi.org/10.1039/c9cc05592d

How to cite this article: Thapa, K., Liu, W., & Wang, R. (2022). Nucleic acid-based electrochemical biosensor: Recent advances in probe immobilization and signal amplification strategies. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*, *14*(1), e1765. https://doi.org/10.1002/wnan.1765