Chinese Chemical Letters 37 (2026) 112451

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

DNAzyme-based sensing tools

Shan Huang, Xinyi Jing, Jin Shao, Xiaoming Ren, Xiaojun Chen*

State Key Laboratory of Materials-Oriented Chemical Engineering, College of Chemistry and Molecular Engineering, Jiangsu Provincial University Key Laboratory of Intelligent Medical Sensing Materials and Devices, Nanjing Tech University, Nanjing 211816, China

ARTICLE INFO

Article history:
Received 2 August 2025
Revised 31 December 2025
Accepted 23 January 2026
Available online 23 January 2026

Keywords:
RNA-cleavage DNAzyme
Peroxidase mimicking DNAzyme
Nanomaterials
Metal ions
Nucleic acid
Bacteria

ABSTRACT

DNAzyme is an artificially created deoxyribonuclease that is produced through *in vitro* screening. Due to the special properties of DNAzyme, such as catalytic activity, recognition function, excellent thermal stability and flexible designability, it has a broad prospect in constructing biosensors. Many innovative biosensors have been developed by integrating DNAzymes with nanomaterials that exhibit distinct physicochemical properties. This review focuses on the classification and application of DNAzyme-based biosensors, such as RNA-cleavage DNAzyme (8-17, 10-23 DNAzyme), peroxidase mimicking DNAzyme. Finally, some risks and challenges in this field are also discussed.

© 2026 Published by Elsevier B.V. on behalf of Chinese Chemical Society and Institute of Materia Medica, Chinese Academy of Medical Sciences.

1. Introduction

Nucleic acids are functional macromolecular biomolecules capable of storing genetic information. Functional nucleic acids represent a class of biomolecules with specialized biological activities, primarily including DNAzymes (DNA enzymes) and DNA aptamers. DNAzymes were initially identified by Breaker and Joyce in 1994 by means of *in vitro* selection techniques, although they were not naturally occurring. These synthetic catalytic DNA molecules have since gained widespread application due to their robust and programmable enzymatic activity [1–4]. Furthermore, using repetitive selection and amplification cycles, aptamers are usually obtained from randomized DNA sequence libraries. In this procedure, the separation of high-affinity nucleic acid ligands that bind particular molecular targets with remarkable selectivity is made possible by the known as Systematic Evolution of Ligands by EXponential enrichment (SELEX) [5]. DNAzymes are catalytic DNA molecules capable of mediating specific biochemical reactions, such as the cleavage of RNA phosphodiester bonds through enzymatic activity. In contrast, DNA aptamers are structured nucleic acids that fold into precise three-dimensional conformations, enabling high-affinity and selective binding to target molecules, including proteins and small molecular ligands. While both belong to the functional nucleic acid family, they exhibit fundamentally distinct molecular mechanisms: DNAzymes demonstrate catalytic functionality, whereas DNA aptamers perform molecular recognition. Due to these unique properties, these molecules have found extensive applications as powerful tools in the detection and analysis of diverse biological macromolecules.

DNAzymes are catalytically active single-stranded DNA molecules that catalyze the conversion of specific substrate strands and cleavage of RNA bases with the help of cofactors, including different amino acids and metal ions. Moreover, DNAzyme also acts as a catalyst for many biological and chemical reactions [6,7]. As one example, DNAzymes serve as a compelling example of single-stranded DNA molecules exhibiting catalytic activity. Functioning similarly to protein-based enzymes such as nucleases and proteases, these DNA catalysts demonstrate the ability to drive specific chemical reactions, particularly in the presence of metal ion cofactors [8]. Some DNAzymes were selected as catalysts to catalyze various bio-transformations, such as DNA cleavage [9], RNA cleavage [10], RNA ligation [11]. Researchers have found that DNAzyme shows more stable catalytic activity compared with protease and nuclease, as well as simple synthesis, flexible design, low costs and high reactivity, making them available as a potential clinical diagnostic tool for on-site testing.

It is found that DNAzyme are not expressed in their initial primitive stages with catalytic activity, they are activated upon binding with specific targets and then can cleave the corresponding substrates, resulting in detectable signals [3,12]. Typically, DNAzyme consists of a substrate strand and the matching enzyme strand by the Watson-Crick base complementary pairing principle. The DNAzyme system consists of two key components, a substrate strand containing a single riboadenosine (rA) cleavage site, and an enzyme strand featuring a ~15-nucleotide catalytic core flanked by

* Corresponding author.
E-mail address: chenxj@njtech.edu.cn (X.J. Chen).

https://doi.org/10.1016/j.cclet.2026.112451
1001-8417/© 2026 Published by Elsevier B.V. on behalf of Chinese Chemical Society and Institute of Materia Medica, Chinese Academy of Medical Sciences.

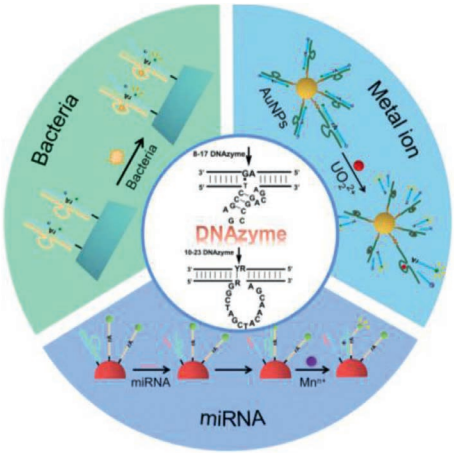


Fig. 1. Working principle diagram DNAzyme-based sensing tools.

two binding arms (typically ~10-nucleotides each) that facilitate target recognition and binding [13]. The ion-dependent DNAzyme developed and extensively applied so far include Pb^{2+}, Zn^{2+}, Mg^{2+}, Cu^{2+}, $UO_2{}^{2+}$ and Na^{+}-specific recognition structures [14–19]. In the presence of metal ions, the catalytic core chains are expected to bind the metal ions and cleave the substrate chains, prompting the development of DNAzyme as recognition elements for highly sensitive and specific detection of metal ions.

Among the DNAzyme structures, 8-17 and 10-23 DNAzymes are two of the broadest in applications (Fig. 1). Due to their high flexibility and excellent substrate recognition specificity, it is a perfect choice as ion recognition elements and signal amplifiers. In particular, one of the most used RNA-cleavage DNAzyme is the 8-17 DNAzyme. It is obtained by cloning and sequencing a molecule after initial *in vitro* screening in rounds 8 and 10, and then selecting the 17^{th} clone from round 8 [20]. Another highly specific RNA-cleaving DNAzyme is the 10-23 DNAzyme. This molecule, identified as the 23^{rd} clone from the 10^{th} round of *in vitro* selection, demonstrates remarkable catalytic efficiency in mediating RNA cleavage reactions across diverse substrates.

Peroxidase mimicking DNAzyme is another significant functional nuclease. Peroxidase mimicking DNAzyme contains guanine nucleic acid sequences with high thermal stability which is valuable for the development of functional nucleic acids. The G-rich tetramer forms a tetramer shape by stacking in the presence of K^{+}, Na^{+}, $NH_4{}^{+}$ or hemin, allowing it exhibits mimic peroxidase activity for sensors construction [21–25].

Differing from other published reviews that perform highly comprehensive summaries of DNAzymes in molecular recognition-based portable sensors, this review adopts a broader perspective, focusing on the application of DNAzymes in constructing diverse types of sensors [26]. It further expands the scope of application targets and encompasses a richer array of application fields. Additionally, while previous review articles have extensively covered DNAzyme-based biosensing platforms, the present work specifically highlights recent advancements in multiplexed detection methodologies, with particular emphasis on their synergistic integration with functional nanomaterials and miniaturized portable detection systems. Finally, we summarize and discuss the development challenges and opportunities for DNAzyme-based sensing tools.

2. Classification of DNAzyme-based biosensors

All biosensors created to date have been composed of two essential yet fundamental parts: A signal detection component and a molecular recognition element that binds to the target molecule only in specific ways. In the construction process of traditional biosensors, proteins are generally used. However, proteins are challenging to be modified with different ligands for obtaining precise detection when different target analytes need to be detected simultaneously and quickly [27]. DNAzymes, as a unique category of functional nucleic acids, hold promise for use in the medical field as detection tools or recognition elements in biosensors, owing to their inherent properties. DNAzyme can be easily modified and functionalized to imitate a variety of fundamental characteristics of related enzyme-like functions, which is helpful to catalyze various reactions, and enable the quick detection for several targets [28–30].

Nowadays, there are two main categories of biosensors constructed with DNAzyme as the originals. One is the RNA- cleavage DNAzyme sensors relying on their enzyme-like activities to generate signals [31]. In order to increase detection and catalytic efficiency, the researchers are aware of the flexibility of the RNA-cleaved DNAzyme designand the ability to construct the DNAzyme-related sequence in accordance with various target analytes in a flexible manner. As a result, RNA-cleavage DNAzyme has been chosen to be coupled with nanomaterials or other metallic compounds for the construction of biosensors with high detection sensitivity. The other category is peroxidase-mimicking DNAzymes, a class of engineered single-stranded DNA oligonucleotides that demonstrate catalytic activity similar to natural peroxidases [32–34]. In this following section, we will discuss the composition of biosensors utilizing the above two types of DNAzyme as the original components.

RNA- cleavage DNAzyme is an enzyme that precisely breaks down RNA in accordance with its own sequence, andit mostly includes two types of 8-17 and 10-23 DNAzyme [35]. Due to their high metal ion recognition specificity, these DNAzymes are frequently utilized as metal ion detection components. As an instance, the 8-17 DNAzyme depends on the activation of divalent metal ions for its detection capability [36–38]. In contrast to the poor signaling and lack of reactivity towards monovalent ions, 8-17 DNAzyme reacts with divalent metal ions with rate constants up to 200,000-fold like Pb^{2+}. Comparison of the reactivity of 8-17 DNAzyme towards divalent ions and sequential *in vitro* screening eventually determined that 8-17 DNAzyme is a strong tool for very sensitive detection of Pb^{2+} [39]. Meanwhile, the 10-23 DNAzyme, named for its *in vitro* selection process, is developed as a highly specific catalytic DNA molecule capable of ligating any purine-pyrimidine pair [40]. Remarkably, 10-23 DNAzyme can be recognized upon incredibly small changes in the target sequence with extremely high detection sensitivity. Additionally, it also can be employed as a recognition origin for a wide range of mRNAs and viral RNAs and has a broad spectrum of targets. Therefore, choosing 10-23 DNAzyme as a biosensing unit is also a favorable pathway [12,41,42].

Peroxidase-mimicking DNAzymes, as a significant class of DNAzymes, play a crucial role in biology due to their horseradish peroxidase (HRP)-like activity. These DNAzymes have been widely applied in biosensing, bioanalysis, and biomedical fields [43]. G-quadruplexes (G4) are non-canonical nucleic acid structures formed through the planar stacking of guanine (G)-rich sequences. These stable tertiary structures are stabilized by Hoogsteen hydrogen bonding between guanine tetrads and coordination with monovalent cations (particularly K^{+} or Na^{+}) positioned at the central channel. These structures exhibit high thermal stability and biological significance, influencing gene regulation and contributing to genomic instability, hereditary diseases, and cancer progression [44–46]. Although G4-based DNAzymes are well-studied, other hemin-dependent DNAzymes have also been developed. Hemin, a porphyrin cofactor that contains iron, demonstrates inherent

peroxidase-like activity. Notably, it can effectively catalyze peroxidase reactions even without the presence of natural enzymes [47].

3. Applications of DNAzyme-based sensing tools

DNAzymes have garnered significant research interest owing to their unique combination of catalytic activity, molecular recognition capability, and programmable design [48]. Recent advances have revealed additional merits beyond these intrinsic properties: DNAzymes can be chemically and functionally modified, and precisely assembled on electronic sensor surfaces or nanoparticle matrices [49,50]. DNAzymes are made possible by the ability of these features to act as strong molecular recognition components and effective signal amplifiers for sensitive analyte detection. Notably, DNAzymes exhibit exceptional stability in complex biological environments [51], demonstrating superior resistance to degradation compared to protein enzymes and other nucleases. These advantageous characteristics have motivated extensive exploration of DNAzyme-based biosensor development [52]. So far, DNAzyme probe systems have been effectively utilized for the detection of various analytes, including metal ions [53], nucleic acids [54], proteins [55], and bacterial targets [56], among others.

DNAzymes are now more widely used than standard biosensor development thanks to recent developments. The integration of DNAzymes with nanomaterials has emerged as a powerful strategy in biosensing, leveraging the unique advantages of nanostructures including high surface-to-volume ratios, tunable surface chemistry, excellent biocompatibility, and structural stability. Nanomaterials further contribute unique optical, magnetic, and electronic properties that enable signal amplification [57,58]. Particularly, the conjugation of nanomaterials with DNAzymes has demonstrated enhanced signal modulation capability and improved cellular transfection efficiency [59]. These hybrid systems serve as versatile building blocks in biosensor design, functioning as sensitive probes and efficient transduction elements that significantly improve detection sensitivity [60,61]. These biosensors can be systematically classified according to their signal transduction mechanisms into five principal categories: fluorescent, colorimetric, electrochemical, photoelectrochemical biosensors, along with other emerging detection modalities [62]. The most widely utilized functional nanomaterials comprise quantum dots (QDs) [63], gold nanoparticles (AuNPs) [64], graphene oxide (GO) [65], magnetic beads (MBs) [36], and carbon nanoparticles (CNPs) [66], each offering distinct advantages for specific sensing applications. Notably, AuNPs have gained particular prominence due to their distinctive plasmonic properties, facile synthesis with controllable size and morphology, exceptional surface-to-volume ratios, and superior biocompatibility [39,67]. For instance, a biofluorescent sensing probe was created by conjugating an RNA-cleaving DNAzyme to AuNPs for the detection of MDA-MB-231 breast cancer cells (Fig. 2A). Its limit of detection (LOD) reached 1.72×10^{-2} pg/mL, demonstrating significant potential for developing ultra-sensitive and rapid fluorescent immunoassay diagnostic platforms [68]. Additionally, DNAzyme-functionalized MBs constitute an advanced biosensing platform for ultra-sensitive molecular detection. When combined with inductively coupled plasma mass spectrometry (ICP-MS), this hybrid system showcases the ability to quantify multiple miRNAs simultaneously. The assay achieves remarkable detection sensitivity, with LOD spanning 11-20 pmol/L across three target miRNAs (Fig. 2B). This approach establishes a robust methodology for simultaneous, high-sensitivity detection of multiple nucleic acid biomarkers and other molecular targets [69].

In the following parts, we will systematically review the applications of DNAzyme-based sensing tools, categorized according to their target analytes, focusing on: (1) Metal ion detection [62], (2) nucleic acid analysis [65], and (3) bacterial identification [70], reflecting their essential functions in health monitoring, diagnostic medicine and epidemiological safety.

For example, a novel biosensing platform utilizing nickel/iron layered double hydroxide (Ni/Fe-LDH) nanosheets integrated with G4 DNAzyme for label-free Cu^{2+} detection was developed. The sensing mechAanism involves: (1) Assembly of biotinylated Cu^{2+}-specific DNAzyme (Cu^{2+}-enz) with substrate strand (Cu-sub) containing G-rich sequences, (2) Cu^{2+}-dependent catalytic cleavage releasing G-rich fragments, (3) K^{+}-stabilized G4 formation, and (4) magnetic bead-based separation of reaction components. The Cu^{2+}-triggered self-assembly between G4 structures and Ni/Fe-LDH nanosheets significantly enhances peroxidase-mimicking activity, achieving an exceptional detection limit of 0.2 nmol/L in human serum samples [71].

And a groundbreaking photocage-assisted detection framework was developed, comprising three key innovations: (1) XDPAdeCage-mediated Zn^{2+} chelation, (2) light-controlled liberation from serum biomatrix, and (3) 8-17 DNAzyme-based fluorescent readout. The approach significantly enhances detection affordability (40% cost reduction) and user-friendliness, offering transformative potential for point-of-care biosensor development [72].

Currently, the application of DNAzyme biosensors for metal ion detection *in vitro* has been well established, while research on biosensing technology for real-time monitoring of metal ions in living cells is achieving groundbreaking progress and has already contributed to the medical field. The first DNAzyme-based metal ion sensor for living cells was constructed by coupling DNAzyme with fluorescent group-modified AuNPs [73]. Subsequently, by integrating DNAzyme with AuNPs, researchers developed a target-triggered, Cu^{2+}-dependent DNAzyme walker for highly sensitive detection of intracellular Cu^{2+} (Fig. 3A). Compared with conventional DNAzyme walkers, this Cu^{2+}-specific walker demonstrates significantly enhanced binding affinity and cleavage efficiency, achieving a detection limit as low as 3 nmol/L with a remarkably faster assay time of 40 min [74]. Consequently, DNAzyme-based fluorescent probes for imaging metal ions in living cells have emerged as a research hotspot. A dual-switch DNAzyme system incorporating clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 and 470 nm photoactivation was successfully developed. This strategy further demonstrated its superior spatiotemporal imaging capabilities by integrating the CRISPR-DNAzyme system with photoactivation approaches and Boolean logic gates, enabling dynamic monitoring of nuclear Zn^{2+} in both HeLa cells and mouse models (Fig. 3B) [75].

A nanoplatform co-assembled from DNAzyme and UCNPs enables mitochondria-targeted Zn^{2+} imaging in living cells through near-infrared (NIR) light-controlled activation, which specifically releases organelle-localizing signals (Fig. 4A) [76]. This advancement significantly expands the repertoire of detectable metal ions in intracellular imaging applications. Subsequently, for the simultaneous detection strategy of multiple metal ions in cells, an intelligent acid-switchable nanodevice based on pH-responsive DNA strands and DNAzyme precursors was developed [77]. This device, constructed from DNAzyme precursors (DPs) and acid-switchable DNA strands (SW-DNA), successfully achieved *in situ* simultaneous detection of Zn^{2+} and Pb^{2+} (Fig. 4B).

The following year, researchers successfully developed two breakthrough DNAzyme detection systems: (1) An intelligent nanodevice based on pH-responsive SW-DNA and DPs that can be activated by acidic environments within living cells to achieve *in situ* synchronous imaging of Zn^{2+}/Pb^{2+}; and (2) a chemiluminescent resonance energy transfer (CRET) fluorescence sensor incorporating FAM-labeled DNAzyme and hemin/G4 quadruplex, enabling Pb^{2+} detection at 5 nmol/L level without requiring external light excitation, which has been extended to smartphone-based portable environmental monitoring applications. These innovations

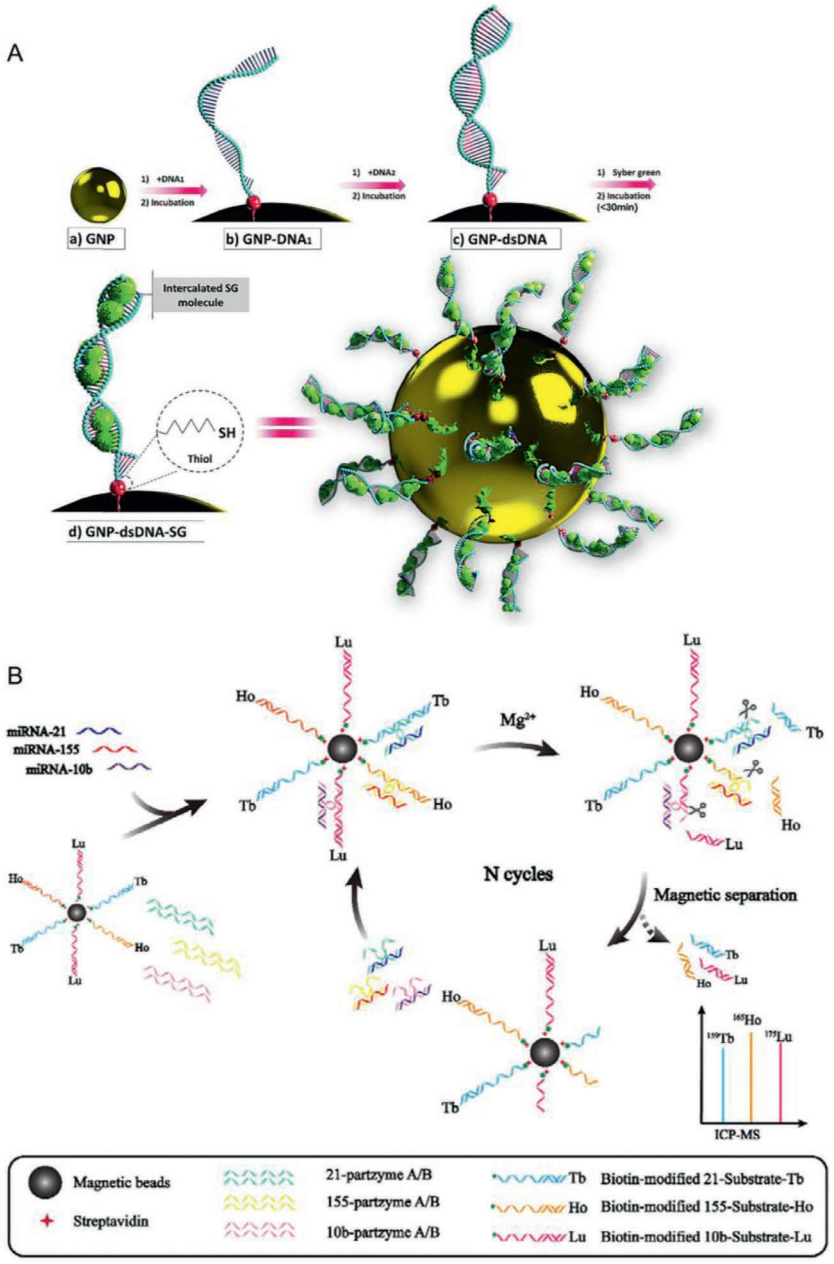


Fig. 2. (A) RNA-cleaved DNAzyme modification on GNP for the detection of MDA-MB-231 breast cancer biomarkers. Reproduced with permission [68]. Copyright 2023, Royal Society of Chemistry. (B) Schematic representation of multiple miRNA detection by MNAzyme-ICP-MS. Reproduced with permission [69]. Copyright 2021, American Chemical Society.

provide novel solutions for intracellular multi-ion analysis and on-site rapid detection [78].

We also discussed in detail the specific contents of nucleic acid testing, including functional DNA, RNA and miRNA. For instance, a novel DNAzyme hydrogel platform enables smartphone-based visual detection of cfDNA. This system utilizes G4-catalytic DNA hydrogels to drive chromogenic reactions, achieving a detection range of 0.1 pmol/L-1500 nmol/L with a sensitivity of 0.042 pmol/L and demonstrating high recovery rates in serum testing. Although slightly less sensitive than conventional methods, its advantages in portability and cost-effectiveness establish a foundation for intelligent diagnostics [79]. Researchers have also successfully developed DNAzyme probes integrated with nanomaterials. The system utilizes AuNPs functionalized *via* Au–S bonds with catalytic DNAzyme subunits and shared substrates, enabling target-dependent activation of the silenced DNAzyme and subsequent fluorescence signal recovery through substrate cleavage. This approach achieves ultrasensitive detection (LOD, 50 fmol/L) of programmed death-ligand 1 (PD-L1) RNA in mesothelial cell lines, rapid response (8 min) under isothermal conditions and protease-free operation [80].

The unique structural features of miRNAs, including their short chain length, small size, low abundance, and high sequence homology, necessitate an exceptionally high level of sensitivity in the development of biosensors for their detection [81]. In 2024, researchers introduced a portable, enzyme-free miRNA detection platform combining target recycling and entropy-driven DNA assembly (EDA) [82]. The innovative hairpin probe (H-probe) design allows target-triggered cyclic amplification, EDA-mediated signal enhancement and sucrose-to-glucose conversion quantifiable by personal glucose meter (PGM) achieving dual amplification (LOD, 2.54 fmol/L), enzyme-independent operation and point-of-care compatibility while circumventing complex primer requirements,

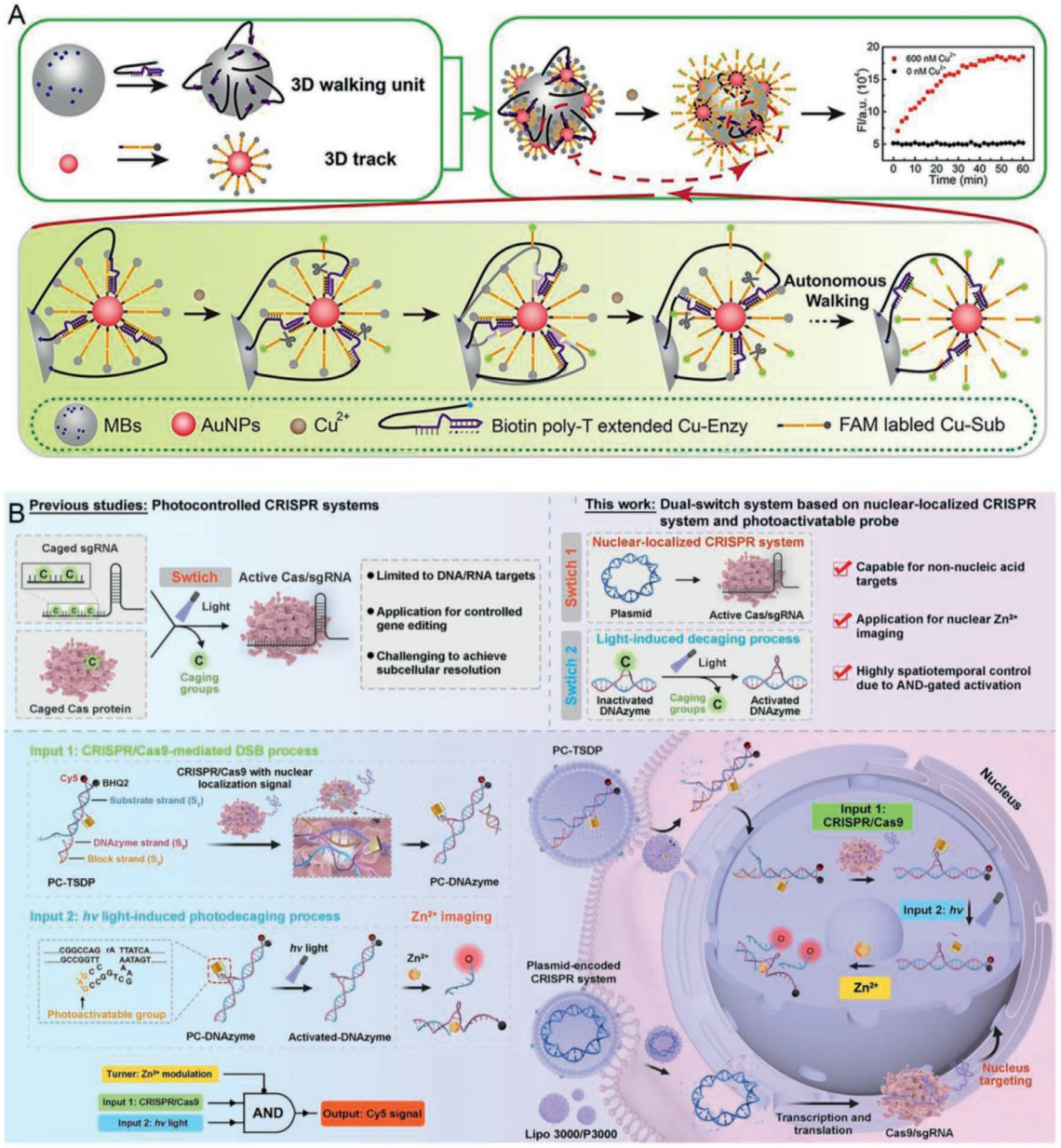


Fig. 3. (A) Schematic of a target-triggered Cu^{2+}-dependent DNAzyme walker with a 3D walking device. Reproduced with permission [74]. Copyright 2021, Elsevier. (B) Illustration of a photoactivatable CRISPR/Cas9 inducible DNAzyme probe designed for nuclear Zn^{2+} imaging. Reproduced with permission [75]. Copyright 2024, Wiley-VCH.

offering strong clinical translation potential for miRNA diagnostics. DNAzymes can also serve as detection probes in electrochemiluminescence (ECL) applications. Specifically, ECL biosensor combines a miRNA-activated DNAzyme switch (OFF to ON) with NIR emission-emitting NS-CDs, enabling ultrasensitive miRNA-155 detection (58.5 amol/L) through dual-recognition while minimizing photodamage *via* low-potential NIR ECL, showing great clinical diagnostic potential (Fig. 5A) [83]. Notably, the strategic combination of DNAzymes with engineered nanomaterials has become pivotal in enhancing the performance of fluorescence-based biosensors, particularly through improved signal amplification and target recognition. Researches developed a streamlined isothermal amplification system combining toehold-mediated strand displacement with localized DNAzyme cascades, achieving cascade-network-level efficiency without complex architectures. Notably, the strategic incorporation of superparamagnetic Fe_3O_4@SiO_2 nanoparticles provides dual advantages: (1) Significantly enhanced detection sensitivity (84 zmol/L for miRNA-122, 8-log improvement) through improved DNAzyme positioning efficiency, and (2) accelerated detection kinetics (<15 min) enabled by rapid magnetic separation of DNA-CdTe quantum dot probes. This nanomaterial-enhanced system maintains excellent specificity, broad temperature adaptability (20-60 °C), and low cost (<$0.5/test), demonstrating particular promise for point-of-care diagnostics (Fig. 5B) [84]. In recent years, the introduction of DNAzymes into enzyme biofuel cell-based self-powered biosensor (EBFC-SPB) has further enhanced the detection sensitivity of these sensors. For example, an innovative EBFC-SPB that integrates a DNAzyme Walker with dumbbell hybridization chain reaction (DHCR) for dual-amplified detection of the thalassemia biomarker TATA-28 at an ultralow detection limit of 35.3 amol/L. The system employs an Au@Zr-MOF/graphdiyne hybrid substrate, where target-activated DNAzyme initiates the release of S0 strand to trigger DHCR amplification, subsequently forming electroactive DNA nanostructures that efficiently adsorb

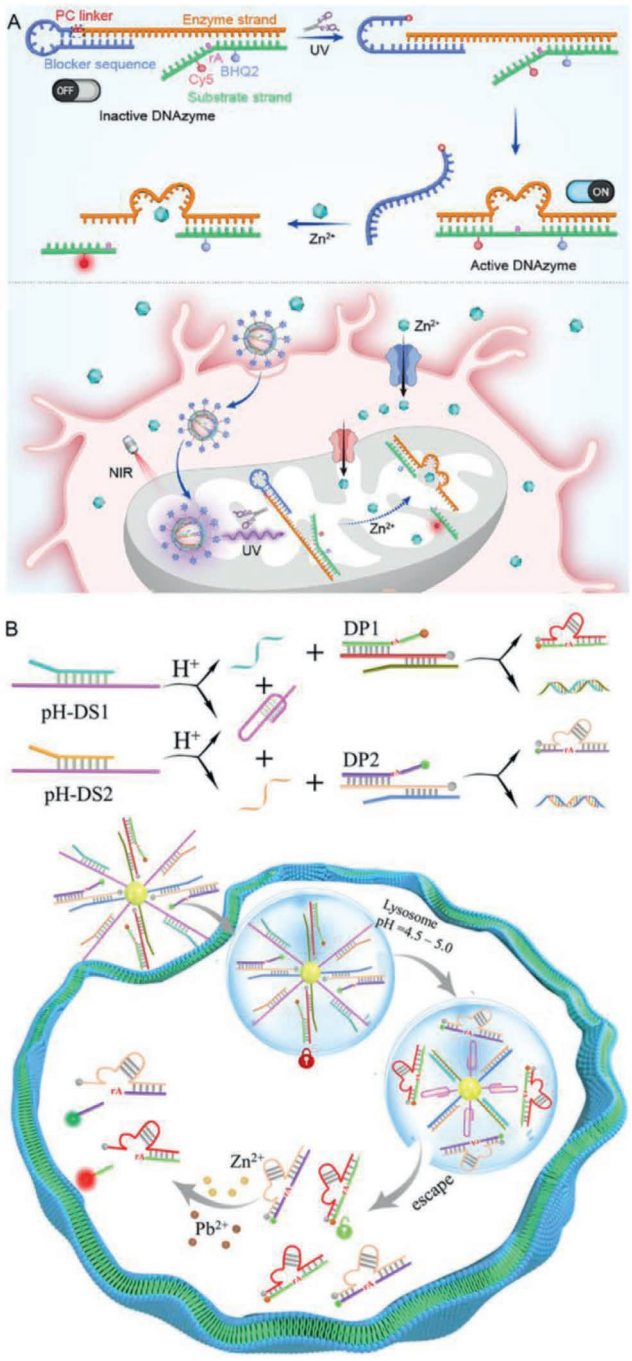


Fig. 4. (A) DNAzyme-assembled core-satellite structure for imaging and detecting Cu^{2+} in living cells. Reproduced with permission [76]. Copyright 2023, American Chemical Society. (B) TP fluorescent DNAzyme probe immobilized on AuNPs for detection of Zn^{2+} in living cells. Reproduced with permission [77]. Copyright 2020, American Chemical Society.

$[Ru(NH_3)_6]^{3+}$ and significantly enhance the open-circuit voltage signal. This biosensor showcases outstanding analytical performance, offering a broad linear range from 0.1 fmol/L to 10 nmol/L and achieving satisfactory recovery rates between 90.1 % and 106.5 % in human serum samples, making it a reliable and sensitive option for early thalassemia screening in clinical diagnostics (Fig. 5C) [85].

Traditional bacterial detection methods, including culture-based assays and enzyme-linked immunosorbent assay (ELISA), suffer from prolonged turnaround times (typically 24-72 h) and operational complexity, rendering them inadequate for rapid response in public health emergencies such as foodborne outbreaks and nosocomial infections. To overcome these limitations, researchers have developed next-generation rapid microbial sensing technologies. Of them, a groundbreaking multiplex polymerase chain reaction-surface-enhanced Raman spectroscopy (PCR-SERS) platform demonstrates particular promise, enabling simultaneous detection of four clinically relevant pathogens, *pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus*, *S. epidermidis* (both Gram-positive), and *Candida lusitaniae* (fungal). This innovative strategy addresses the critical sensitivity-throughput trade-off inherent in conventional methods, achieving ultrahigh sensitivity (LOD, 1-10 CFU/mL), multiplexing capacity for parallel pathogen detection, and rapid turnaround (<2 h), representing a 12-fold improvement over standard protocols [86,87].

Equally promising in detection sensitivity are hybrid CRISPR/Cas12a-DNAzyme biosensors, which further expand the toolbox for rapid, precise bacterial identification. This study developed a novel detection system by engineering blocking crRNA (bcrRNA) and Mn^{2+}-mediated Cas12a regulation. Target bacteria capture triggered MnO_2 etching to release Mn^{2+}, which activated DNAzyme to cleave bcrRNA and restore CRISPR/Cas12a activity, enabling nucleic acid extraction-free ultrasensitive detection (5 CFU/mL) within 29 min. Notably, this versatile platform can be adapted for other targets by replacing recognition elements, demonstrating broad application potential [88]. The high specific surface area provided by nanomaterials in the aforementioned work serves as a prerequisite for achieving high sensitivity, while other detection advantages associated with nanomaterials continue to be developed. For instance, a microfluidic system based on RNA-cleavage DNAzymes enables real-time, culture-free monitoring of *Legionella pneumophila* in cooling tower water (Fig. 6A). This system incorporates RCD-functionalized microgel MB that release electroactive DNA markers upon pathogen recognition, facilitating real-time electrochemical detection. Through quantitative analysis of key parameters including peak current, signal slope, and lag time, the system achieves detection limits of 1.4×10^3 CFU/mL in buffer and 1.9×10^3 CFU/mL in cooling tower water samples, meeting regulatory requirements. This approach provides a practical solution for on-site monitoring applications [89]. Meanwhile, single-walled carbon nanotubes (SWCNTs) have also been incorporated with DNAzymes for sensor development. The system specifically recognizes *Shigella flexneri* through HGD-SWCNT complex formation, generating visible colorimetric signals *via* enzymatic reactions. With a detection limit of 51 CFU/mL, the method demonstrates excellent specificity in complex bacterial environments without cross-reactivity. This simple, rapid, and cost-effective detection strategy shows strong potential for applications in food safety, environmental monitoring, and public health surveillance [90]. Moreover, leveraging the electrochemical specificity of metal-organic framework (MOF) materials, they can serve as an ideal carrier for integration with DNAzymes, enabling dual-signal detection of target analytes. They engineered a novel ratio-type electrochemical biosensor for highly sensitive Salmonella detection, integrating DNAzyme signals with MOF through target-triggered catalytic hairpin assembly (CHA) [91]. The system specifically recognizes Salmonella typhimurium (ST) *via* DNAzyme-mediated RNase H2 (STH2) release, which cleaves the rA site to initiate CHA amplification. Employing ferrocene (Fc)-labeled H1 DNA as the signal tag and Fe-MOF as an internal reference, we established a robust dual-signal output strategy (Fig. 6B). Validation using urban water samples demonstrated superior sensitivity to commercial test strips while maintaining excellent accuracy, confirming its practical utility.

Building upon these DNAzyme-based microbial sensors, several promising research directions are emerging as current hotspots for in-depth investigation including microfluidic-integrated plat-

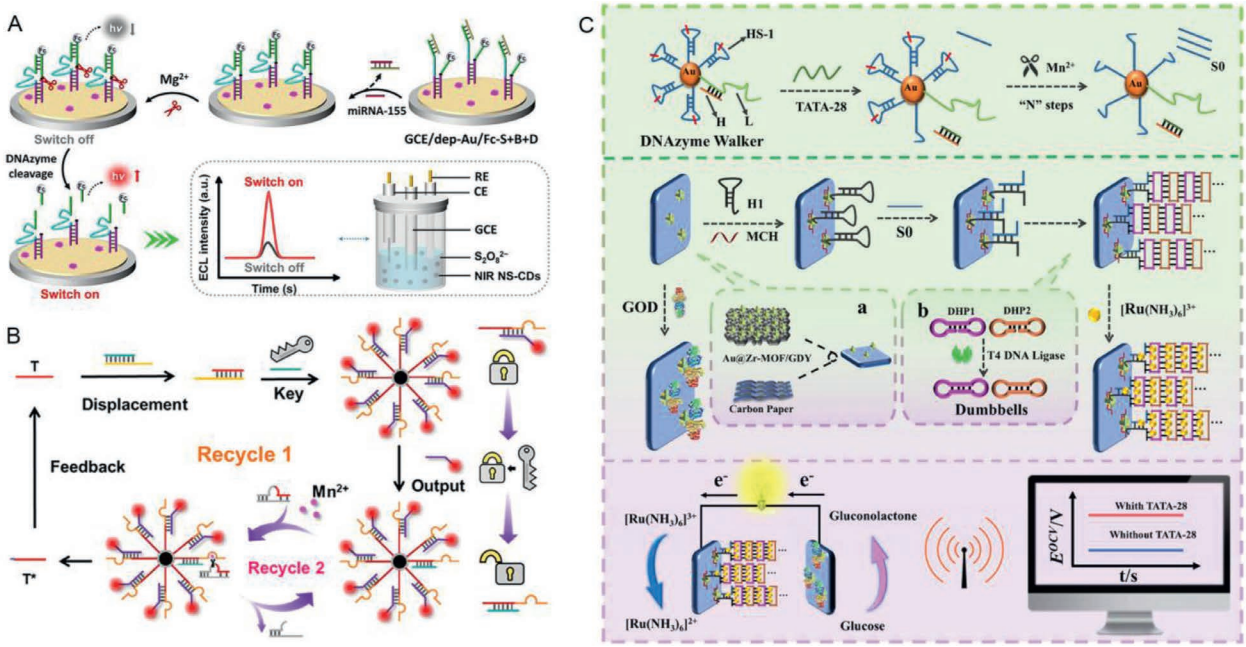


Fig. 5. (A) Schematic diagram of the construction of an electrochemical luminescence biosensor based on DNA enzymes for the detection of miRNA-15. Reproduced with permission [83]. Copyright 2025, American Chemical Society. (B) A schematic diagram of a double-loop DNA enzyme network structure that incorporates a positive self-feedback mechanism for ultra-sensitive fluorescence detection of miRNA-122. Reproduced with permission [84]. Copyright 2025, American Chemical Society. (C) Illustration of the preparation process of self-powered biosensors. Reproduced with permission [85]. Copyright 2025, Elsevier.

forms for point-of-care (POC) testing, artificial intelligence-assisted Raman spectroscopy analysis algorithms, and probe modification strategies to counteract matrix interference (particularly from complex food components). A DNAzyme-based colorimetric hydrogel sensor was modified by the Mann team for the purpose of detecting pathogens in the naked eye. Upon target recognition, DNAzyme-mediated hydrogel dissolution releases encapsulated AuNPs, generating a visible signal. By optimizing hydrogel polymerization and incorporating phage-assisted DNAzyme amplification, the sensor detects *Escherichia coli* at ultralow concentrations (101 CFU/mL) in lake water (Fig. 6C). Integrated AI analysis achieves 96 % true positive and 100 % true negative rates. Demonstrating exceptional selectivity and stability, it accurately identifies *Escherichia coli*-associated urinary tract infections in clinical samples (zero false positives in controls) [92]. Another POC-compatible approach involves the development of a photonic crystal microfluidic biosensor (PC-μchip) integrated with fluorescent DNAzyme (FDz) (Fig. 6D) [93]. Compared to conventional microfluidic technologies, this innovation demonstrates a tenfold improvement in detection sensitivity through fluorescence technology, achieving a detection limit of 100 CFU/mL for *Escherichia coli*. The device ingeniously incorporates three core modules: (1) A serpentine reaction channel, (2) a magnetic bead enrichment unit, and (3) a photonic crystal signal amplification system. When target bacteria are present, FDz releases fluorescent single-stranded DNA (ssDNA) that specifically migrates to the photonic crystal surface for signal enhancement, enabling highly specific and sensitive identification. Method validation using 20 clinical urine samples confirmed complete detection within 55 min, with both sensitivity and specificity reaching 100 %. This system demonstrates significant potential for POC diagnostics, representing a major advancement in DNAzyme-based on-site bacterial detection.

4. Perspectives and outlooks

Unlike naturally occurring nucleases, DNAzymes possess characteristics such as recognition capability, catalytic activity, high selectivity, and design flexibility, making them an ideal choice for constructing biosensors. This review systematically outlines the design principles and engineering strategies behind DNAzyme-based biosensing tools, providing an in-depth discussion of their novel applications in the quantitative detection and analysis of various chemical and biological targets, such as metal ions, nucleic acids, and bacteria. In addition to the primary detection targets discussed above, DNAzyme-based biosensors have demonstrated remarkable versatility in detecting a wide range of analytes, including small-molecule biomolecules and metabolites (*e.g.*, ATP [94], nucleotides [95], and amino acids [96]), as well as macromolecular targets such as proteins [97] and cancer cells [98]. This broad detection capability has made DNAzyme biosensors an increasingly prominent focus of research in recent years.

Firstly, Primary screening-derived DNAzymes, particularly the 8-17 DNAzyme, have found extensive applications in biosensing. However, while demonstrating exceptional specificity toward divalent metal ions, current detection strategies for monovalent metal ions (*e.g.*, Na^+ and K^+) remain limited, with notably stagnant research progress in recent years [99–101]. It should be emphasized that both mono- and divalent metal ions are equally crucial for maintaining physiological homeostasis in biological systems. Consequently, we are confronted with dual challenges: (1) Optimizing existing methodologies for divalent metal ion detection in living cells, while (2) persistently developing novel DNAzyme-based sensing platforms for monovalent ion recognition.

Secondly, current nucleic acid detection methodologies for DNA and mRNA require further refinement and technological advancement. While miRNA detection platforms and biosensor construction techniques have reached relative maturity, several critical challenges persist. These include: (1) The extremely low abundance of cancer-specific miRNAs and dysregulated miRNAs, (2) the presence of homologous miRNA sequences that compromise detection specificity, and (3) the consequent reduction in analytical sensitivity due to cross-reactivity.

Finally, the field of *in situ* detection for biological targets (particularly bacterial species) remains underdeveloped due to several

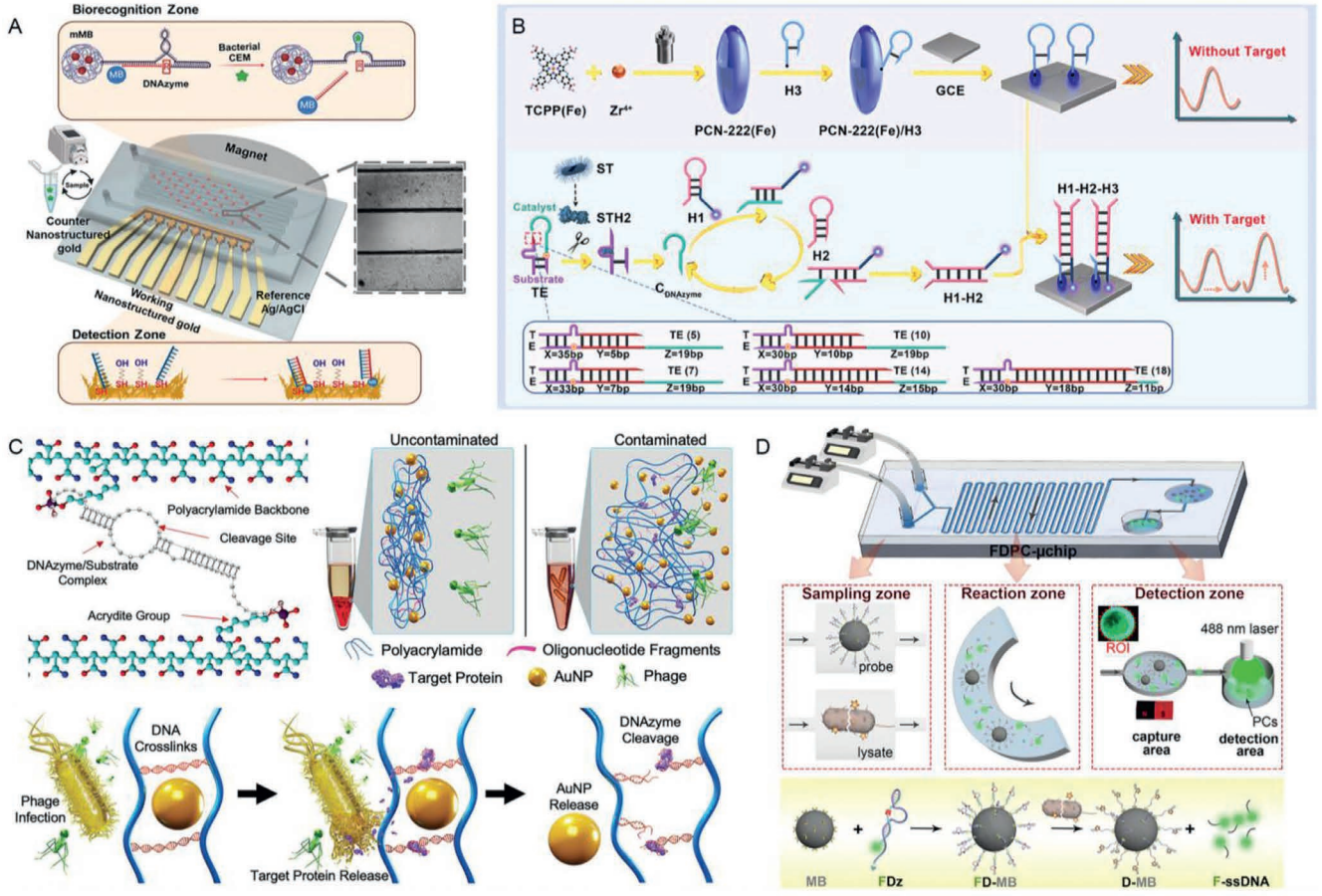


Fig. 6. (A) A schematic diagram of the bio-recognition area integrated in the microfluidic biosensor responding to the target substance by breaking down an electronic barcode. Reproduced with permission [89]. Copyright 2025, Elsevier. (B) Schematic diagram of the preparation process of PCN-222(Fe) and PCN-222(Fe)/H3. The Electrochemical Sensing Platform Based on DNAzyme and Catalytic Hairpin Self-Assembly for Salmonella Detection. Reproduced with permission [91]. Copyright 2025, American Chemical Society. (C) Colorimetric detection of Escherichia coli platform diagram. Reproduced with permission [92]. Copyright 2025. Wiley. (D) Schematic diagram of the working principle of the FDPC-μ chip. Reproduced with permission [93]. Copyright 2025, American Chemical Society.

critical limitations: (1) Only seven DNAzymes have been successfully selected for direct bacterial detection; (2) Just one known DNAzyme demonstrates selectivity for multi-drug resistant bacterial strains; (3) No existing DNAzyme can distinguish between pathogenic and non-pathogenic variants within the same bacterial species; and (4) A severe shortage of target-specific recognition prototypes persists. These fundamental gaps significantly hinder the development of categorical biosensors, necessitating continued exploration of innovative detection technologies to overcome these challenges.

Recent years have witnessed remarkable advances in the development and application of DNAzyme-based sensing tools, demonstrating significant potential for detecting prevalent and clinically important diseases. Nevertheless, further improvements in sensitivity and accuracy remain imperative to enable reliable *in situ* real-time detection of biological samples. Such advancements would provide more robust diagnostic tools and generate fundamental data to support clinical decision-making.

CRediT authorship contribution statement

Shan Huang: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Xinyi Jing:** Writing – original draft, Investigation, Formal analysis, Data curation. **Jin Shao:** Methodology, Investigation, Formal analysis, Data curation. **Xiaoming Ren:** Visualization, Funding acquisition. **Xiaojun Chen:** Writing – review & editing, Visualization, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We greatly appreciate the supports from the National Natural Science Foundation of China (No. 22304080); The Natural Science Foundation of the Jiangsu Higher Education Institutions of China (No. 23KJB150011); the Natural Science Foundation of Jiangsu Province (No. BK20230310); State Key Laboratory of Analytical Chemistry for Life Science (No. SKLACLS2305); State Key Laboratory of Materials-Oriented Chemical Engineering (No. SKL–MCE–24B09).

References

[1] A. Ponce-Salvatierra, P. Boccaletto, J.M. Bujnicki, Nucleic Acids Res. 49 (2021) D76–D81.
[2] R.R. Breaker, G.F. Joyce, Chem. Biol. 1 (1994) 223–229.
[3] X. Chen, X. Fu, Y. Wu, Y. Jin, W. Li, Anal. Methods 12 (2020) 1579–1586.
[4] M. Liu, D.R. Chang, Y. Li, Acc. Chem. Res. 50 (2017) 2273–2283.
[5] Y. Xiang, Y. Lu, Anal. Chem. 84 (2012) 9981–9987.
[6] P. Gillespie, S. Ladame, D. O'Hare, Analyst 14 (2018) 114–129.
[7] Z. Li, J. Wang, Y. Li, X. Liu, Q. Yuan, Mater. Chem. Front. 2 (2018) 423–436.
[8] L. Gong, Z. Zhao, Y. Lv, et al., Chem. Commun. 51 (2015) 979–995.
[9] L. Zhang, Y. Zhang, M. Wei, et al., New J. Chem. 37 (2013) 1252–1257.

[10] S.W. Stephen, G.F. Joyce, K. Sakthivel, S. Gramatikova, C.F. Barbas, J. Am. Chem. Soc. 122 (2000) 2433–2439.
[11] W.E. Purtha, R.L. Coppins, M.K. Smalley, S.K. Silverman, J. Am. Chem. Soc. 127 (2005) 13124–13125.
[12] S.W. Santoro, G.F. Joyce, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 4263–4266.
[13] J. Liu, Z. Cao, Y. Lu, Chem. Rev. 109 (2009) 1948–1998.
[14] J. Liu, Y. Lu, Chem. Mater. 16 (2004) 3231–3238.
[15] R. Orbach, B. Willner, I. Willner, Chem. Commun. 15 (2015) 4144–4160.
[16] Y. Cheng, Y. Huang, J. Lei, L. Zhang, H. Ju, Anal. Chem. 86 (2014) 5158–5163.
[17] J. Liu, Y. Lu, J. Am. Chem. Soc. 129 (2007) 9838–9839.
[18] J.H. Lee, Z. Wang, J. Liu, Y. Lu, J. Am. Chem. Soc. 130 (2008) 14217–14226.
[19] K. Chen, Q. Huang, T. Fu, et al., Anal. Chem. 92 (2020) 7404–7408.
[20] Y. Lin, Z. Yang, R.J. Lake, C. Zheng, Y. Lu, Angew. Chem. Int. Ed. 58 (2019) 17061–17067.
[21] M. Cepeda-Plaza, C.E. McGhee, Y. Lu, Biochemistry 57 (2018) 1517–1522.
[22] J.L. Mergny, D. Sen, Chem. Rev. 119 (2019) 6290–6325.
[23] J. Wang, M. Cheng, J. Chen, et al., Chem. Commun. 56 (2020) 1839–1842.
[24] Z. Tian, C. Zhou, C. Zhang, et al., J. Mater. Chem. B 10 (2022) 5303–5322.
[25] Y. Wan, Y. Zhou, K. Xiao, et al., Chem. Commun. 55 (2019) 3278–3281.
[26] E.M. McConnell, I. Cozma, Q. Mou, et al., Chem. Soc. Rev. 50 (2021) 8954–8994.
[27] S. Gao, R. Wu, Q. Zhang, New J. Chem. 46 (2022) 6775–6782.
[28] S. Khan, B. Burciu, C.D.M. Filipe, et al., ACS Nano 15 (2021) 13943–13969.
[29] K. Schlosser, Y. Li, Chem. Biol. 16 (2009) 311–322.
[30] H. Wang, H. Wang, Q. Wu, et al., Chem. Sci. 10 (2019) 9597–9604.
[31] S.K. Silverman, Nucleic Acids Res. 33 (2005) 6151–6163.
[32] L. Gu, Y. Ding, Y. Zhou, et al., Angew. Chem. Int. Ed. 63 (2024) e202314450.
[33] C. Li, X. Li, S. Wei, T. Wu, et al., Anal. Chem. 97 (2025) 3680–3686.
[34] S. Zhi, M. Lyu, C. Dennis, et al., Chin. Chem. Lett. 30 (2019) 1652–1654.
[35] H. Liu, X. Yu, Y. Chen, et al., Nat. Commun. 8 (2017) 1–10.
[36] W. Li, Z. Zhang, W. Zhou, ACS Sens. 2 (2017) 663–669.
[37] M. Cepeda-Plaza, A. Peracchi, Org. Biomol. Chem. 18 (2020) 1697–1709.
[38] H. Wang, D. He, R. Wu, et al., Analyst 144 (2018) 143–147.
[39] D. Mazumdar, N. Nagraj, H.K. Kim, et al., J. Am. Chem. Soc. 131 (2009) 5506–5515.
[40] K. Tram, P. Kanda, Y. Li, J. Nucleic Acids 2012 (2012) 1–8.
[41] C.R. Dass, P.F. Choong, L.M. Khachigian, Mol. Cancer Ther. 7 (2008) 243–251.
[42] M.J. Cairns, A. King, L. Sun, Nucleic Acids Res. 31 (2003) 2883–2889.
[43] Y. Cao, W. Li, P. Ding, R. Pei, Chem. Commun. 56 (2020) 8667–8670.
[44] C. Li, X. Li, S. Wei, T. Wu, Anal. Chem. 97 (2025) 3680–3686.
[45] Y. Cheng, M. Cheng, J. Hao, et al., Anal. Chem. 93 (2021) 6907–6912.
[46] V.S. Chambers, G. Marsico, J.M. Boutell, et al., Nat. Biotechnol. 33 (2015) 877–881.
[47] L. Gu, Y. Ding, Y. Zhou, et al., Angew. Chem. Int. Ed. 63 (2024) e202314450.
[48] J.M. Boyce, J. Hosp. Infect. 65 (2007) 50 –44.
[49] Y. Jia, F. Li, Anal. Chem. 90 (2018) 5153–5161.
[50] D. Zhu, H. Pei, J. Chao, et al., Nanoscale 7 (2015) 18671–18676.
[51] T. Yao, J.J. Przybyla, P. Yeh, et al., Org. Biomol. Chem. 19 (2021) 171–181.
[52] D. Morrison, M. Rothenbroker, Y. Li, Small Methods 2 (2018) 1–12.
[53] J. Xu, R. Jiang, Y. Feng, et al., Coord. Chem. Rev. 459 (2022) 1–31.
[54] H. Peng, A.M. Newbigging, Z. Wang, et al., Anal. Chem. 90 (2018) 190–207.
[55] Y. Cao, H. Zhang, X.C. Le, Anal. Chem. 93 (2021) 15712–15719.
[56] H. Yousefi, S.E. Samani, S. Khan, et al., ACS Nano 16 (2022) 29–37.
[57] Z. Yuan, C. Hu, H. Chang, C. Lu, Analyst 141 (2016) 1611–1626.
[58] R. Hu, X. Zhang, R. Kong, et al., J. Mater. Chem. 21 (2011) 16323–16334.
[59] Y. Xiang, P. Wu, L.H. Tan, Y. Lu, Adv. Biochem. Eng. Biotechnol. 140 (2014) 93–120.
[60] N. Li, X. Su, Y. Lu, Analyst 140 (2015) 2916–2943.
[61] J. Lei, H. Ju, Chem. Soc. Rev. 41 (2012) 2122–2134.
[62] L. Hu, X. Fu, G. Kong, et al., J. Mater. Chem. B 8 (2020) 9449–9465.
[63] C.S.M. Martins, A.P. LaGrow, J.A.V. Prior, ACS Sens 7 (2022) 1269–1299.
[64] Y. Yang, S. Zhong, K. Wang, J. Huang, Analyst 144 (2019) 1052–1072.
[65] H. Liu, Y. You, Y. Zhu, H. Zheng, Anal. Methods 13 (2021) 5103–5119.
[66] X. Hu, Z. Song, Z. Yang, et al., Analyst 147 (2022) 2615–2632.
[67] G. Schmid, B. Corain, Eur. J. Inorg. Chem. 17 (2003) 3081–3098.
[68] Z. Ghafary, R. Hallaj, A. Salimi, F. Khosrowbakhsh, J. Mater. Chem. B 11 (2023) 1568–1579.
[69] Q. Kang, M. He, B. Chen, G. Xiao, B. Hu, Anal. Chem. 93 (2021) 737–744.
[70] P.H. Agustin, C.C. Foces, S.C. Romero, et al., Nature 586 (2020) 287–291.
[71] Y. Yao, M. Xue, W. Mao, et al., Analyst 146 (2021) 6470–6473.
[72] S. Xing, Y. Lin, L. Cai, et al., S.C. Burdette, Y. Lu, Anal. Chem. 93 (2021) 5856–5861.
[73] P. Wu, K. Hwang, T. Lan, Y. Lu, J. Am. Chem. Soc. 135 (2013) 5254–5257.
[74] L. Zhou, Y. Xiong, H. Wang, et al., Sensors Actuat. B: Chem. 334 (2021) 129589.
[75] R. Liu, D. Jiang, Y. Yun, et al., Angew. Chem. 136 (2024) e202315536.
[76] D. Yi, H. Zhao, J. Zhao, L. Li, J. Am. Chem. Soc. 145 (2023) 1678–1685.
[77] M. Cui, X. Li, J. Xu, H. Chen, ACS Appl. Mater. Interfaces 12 (2020) 13005–13012.
[78] J. Zheng, J. Wai, R. Lake, et al., Anal. Chem. 93 (2021) 10834–10840.
[79] S. Liu, Y. Yang, M. Shi, et al., ACS Sens 7 (2022) 658–665.
[80] S. Zhand, Y. Zhu, H. Nazari, et al., Anal. Chem. 95 (2023) 3228–3237.
[81] Q. Jia, S. Huang, M. Hu, et al., Sens. Actuators B: Chem. 323 (2020) 1–9.
[82] Z. Jiang, Z.Y. Liu, Anal. Chem. 693 (2024) 115593.
[83] H. He, B. Xie, S. Li, et al., Anal. Chem. 97 (2025) 9902–9910.
[84] D. Qian, J. Zhang, Q. Tan, et al., Anal. Chem. 97 (2025) 1620–1626.
[85] N. Feng, Y. Wei, P. Li, et al., Biosens. Bioelectron. 287 (2025) 117683.
[86] N. Lyu, P.R. Potluri, V.K. Rajendran, Y. Wang, A. Sunna, Analyst 140 (2024) 2898–2904.
[87] P. Ge, Y. Wang, J. Hou, et al., Sensor Actua B: Chem. 445 (2025) 138587.
[88] X. Shi, J. Zhang, Y. Ding, et al., Biosens. Bioelectron. 264 (2024) 116671.
[89] E. Osman, S. Saxena, S. Qian, et al., Biosens. Bioelectron. 278 (2025) 117283.
[90] M. Murugan, E. Saranya, M. Ramya, Sci. Rep. 15 (2025) 14942.
[91] Z. Zhu, N. Wang, L. Zhang, et al., Environ. Sci. Technol. 59 (2025) 6512–6520.
[92] H. Mann, S. Khan, A. Prasad, et al., Adv. Mater. 37 (2025) 2411173.
[93] R. Zhang, C. An, J. Li, et al., Anal. Chem. 97 (2025) 16950–16956.
[94] X. Dai, Z. Huang, C. Liu, et al., J. Food Compos. Anal. 139 (2025) 107176.
[95] S.Y. Ouedraogo, M.M.J. Zeye, X. Zhou, et al., Anal. Methods 16 (2024) 5785–5792.
[96] J. Yang, Q. Bi, X. Song, R. Yuan, Y. Xiang, Sensor Actuat. B: Chem. 375 (2023) 132876.
[97] Y. Ma, Y. Ya, Y. Wu, et al., Int. J. Biol. Macromol. 282 (2024) 137536.
[98] Y. Zeng, Y. Guo, J. Liu, R. Yuan, Y. Chai, Anal. Chem. 97 (2025) 14815–14821.
[99] S.F. Torabi, P. Wu, C.M. McGhee, et al., Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 5903–5908.
[100] W. Zhu, Y. Zhang, P. Huang, J. Dong, J. Liu, Nucleic Acids Res. 44 (2016) 354–363.
[101] G. Gao, Y. Cao, W. Liu, et al., Anal. Methods 9 (2017) 5570–5579.