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DNA framework-engineered electrochemical biosensors

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Self-assembled DNA nanostructures have shown remarkable potential in the engineering of biosensing interfaces, which can improve the performance of various biosensors. In particular, by exploiting the structural rigidity and programmability of the framework nucleic acids with high precision, molecular recognition on the electrochemical biosensing interface has been significantly enhanced, leading to the development of highly sensitive and specific biosensors for nucleic acids, small molecules, proteins, and cells. In this review, we summarize recent advances in DNA framework-engineered biosensing interfaces and the application of corresponding electrochemical biosensors.

framework nucleic acids, biosensing interface, electrochemical biosensors, biomarkers, DNA

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Introduction

With the development of DNA nanotechnology, nucleic acids have been harnessed to construct numerous DNA nanostructures with controllable sizes and dimensions (Goodman et al., 2005; He et al., 2008; Kallenbach et al., 1983; Liu et al., 2018c; Qian et al., 2006; Seeman, 2003), including three-dimensional (3D) shells or skeleton DNA frameworks, which are called framework nucleic acids (FNAs) (Ge et al., 2018). These artificial DNA nanostructures offer great potential for interface engineering of nanoscale surface probes, and promote the performance of various biosensors using interface reactions (Ariga et al., 2018; Ben Zion et al., 2017; Edwardson et al., 2016; Fu et al., 2012; Jungmann et al., 2014; Zhang et al., 2018).

For electrochemical DNA (E-DNA) biosensors, the use of FNAs to modify the electrode surface will significantly improve its performance. The 3D FNA-based electrochemical probe system exhibited several unique features. First, FNAs provide a highly programmable approach for engineering the biosensing interfaces of electrodes, which results in a higher number of fabricated probes on the electrode surface (Campuzano et al., 2019; Ge et al., 2019a, 2019b; Kogikoski Jr et al., 2019; Yan et al., 2019). For example, FNAs can be rapidly prepared with high yields, and readily assembled on gold surfaces in an ordered orientation, with well-controlled spacing, and a high level of stability. As a consequence, probes are separated from the electrode surface and the neighboring probes, which improves the kinetics of recognition reaction and the signal to noise (S/N) ratio of the biosensors. Second, FNAs exhibit controllable charge transport properties, which are used to optimize the performance of the FNA-based electrochemical biosensors (Lu et al., 2012). Third, FNA-based electrochemical biosensors exhibited a stronger anti-interference ability and higher protein resistance, when compared with traditional electrochemical sensors, such as single strand DNA functional electrodes. Finally, FNAs provide a platform that can be combined with various materials to further assist the development of electrochemical biosensors. With these ad-

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vantages, many FNA-based electrochemical biosensors have been constructed and applied in various fields (Su et al., 2019; Ye et al., 2018).

In this review, we summarize the current applications of FNA-based electrochemical biosensors, including the detection of nucleic acids, ions, small molecules, proteins, and cells. We also summarize and discuss the challenges of electrochemical biosensors.

FNA-based electrochemical biosensors for nucleic acid analysis

Nucleic acid analysis

It is widely accepted that the occurrence of certain diseases, such as cancer, is accompanied with an abnormal expression of nucleic acids (Bartel, 2009; Chen et al., 2008; Choi et al., 2010; Das and Singal, 2004; Esquela-Kerscher and Slack, 2006; Lu et al., 2005; Pritchard et al., 2012; Rand et al., 2017; Razin and Riggs, 1980). Therefore, sensitive nucleic acid analysis strategies for disease diagnosis have been developed. By taking advantage of FNAs, which have a low cost and a high yield, numerous electrochemical biosensors have been used to detect nucleic acids (Campuzano et al., 2019). In order to improve the recognition ability of target molecules, Pei et al. introduced FNAs to the electrode surface, as shown in Figure 1A (Pei et al., 2010). They found that probes based on DNA tetrahedral nanostructures (TDNs) had enhanced target accessibility, when compared with the traditional probes, such as linear or stem-loop probes. This FNA-based "sandwich-type" E-DNA biosensor consisted of three elements, i.e., capture probes, target molecules, and signal probes, and gave a limit of detection (LOD) of 1 pmol L^{-1} in DNA analysis.

Using FNA-based "sandwich-type" E-DNA biosensors, Wen et al. achieved a sensitive and specific analysis of the polymerase chain reaction (PCR) amplicons of the *E. coli* genome, with an LOD of 10 fmol L^{-1} for synthetic DNA targets, and 0.2 pg μL^{-1} for the *E. coli* genome, through the PCR process (Wen et al., 2016). Zeng et al. simultaneously reported analysis of four pancreatic carcinoma (PC)-related miRNAs, including miRNA21, miRNA155, miRNA196a, and miRNA210, with an LOD of 10 fmol L^{-1} (Zeng et al., 2017). Meanwhile, Dong et al. achieved detection of the H7N9 virus by using the FNA-based "sandwich-type" E-DNA biosensor to identify specific gene sequences. This biosensor can specifically identify the influenza A (H7N9) virus from similar influenza viruses with an LOD of 100 fmol L^{-1} (Dong et al., 2015). By combining a molecular threading-dependent transport system with the "sandwich-type" E-DNA biosensor, Ye et al. reported a single-step electrochemical DNA detection strategy with a sensitivity of one picomole in 60 min (Ye et al., 2019).

Abi et al. constructed a robust electronic switching nanodevice with FNAs on the electrode surface, known as a "nanoswitching-type" E-DNA biosensor as shown in Figure 1B (Abi et al., 2014). In this research, one edge of the tetrahedral DNA framework was designed to recognize the target DNA, while the ferrocene electrochemical tag was made on the reconfigurable edge of the TDNs to report signals. With this "nanoswitching-type" E-DNA biosensor, Li et al. conducted nucleic acid analysis in the range of 1.0 to 500 fmol L^{-1}, based on the electrochemiluminescence (ECL) properties of CdTe nanocrystals, which were enhanced by the gold nanodendrites (Au NDs) in this system (Li et al., 2018b).

Li et al. reported an FNA-based "swing-type" electrochemical biosensor, as shown in Figure 1C. This biosensor contained a long flexible single-strand DNA probe at the vertex of the TDNs, which bonded with the bottom DNA strands differently when targets were absent or present. Using this biosensor, the detection of picomolar DNA in complex systems can be completed in one step within 10 min (Li et al., 2018a).

An electrochemical "molecular beacon-type" E-DNA biosensor, employing FNAs and molecular beacon structures, was constructed by Lin et al.; this is also known as the third generation E-DNA biosensor, as shown in Figure 1D (Lin et al., 2014). In this work, the LOD of a specific miRNA (miRNA-141) target reached 1 fmol L^{-1}. The stem-loop structure plays a key role in increasing specificity and decreasing the background signal.

The detection performance of FNA-based biosensors can be further improved through systematic evaluation. For instance, Lin et al. exploited several types of different-sized FNAs (TDN-7, TDN-13, TDN-17, TDN-26, and TDN-37) to control the distance and density of probes on the electrode surface, as shown in Figure 1E (Lin et al., 2015). The results demonstrated that the lateral spacing and interactions adjusted by the FNA size affected the hybridization time and the hybridization efficiency, resulting in different limits for DNA detection spanning over four orders of magnitude. Later, Song et al. reported that the binding affinity of FNA-based biosensors can be adjusted by inserting effector sequences into the FNA structure, as shown in Figure 1F (Song et al., 2016). In this work, they demonstrated that the adjustable biosensing interface can programmably regulate the LOD of FNA-based biosensors, and the working range can be adjusted up to 100 times.

Han et al. constructed an E-DNA biosensor based on DNA origami for the analysis of miRNA. Compared with FNA-based biosensors, the DNA origami-based biosensor can contain more multiple probes that extend from the surface. Using methylene blue as the signal reporter, the LOD of the origami-based biosensor reached 79.8 fmol L^{-1}, with an analysis range of 0.1 pmol L^{-1} to 10.0 nmol L^{-1} (Han et al., 2019).

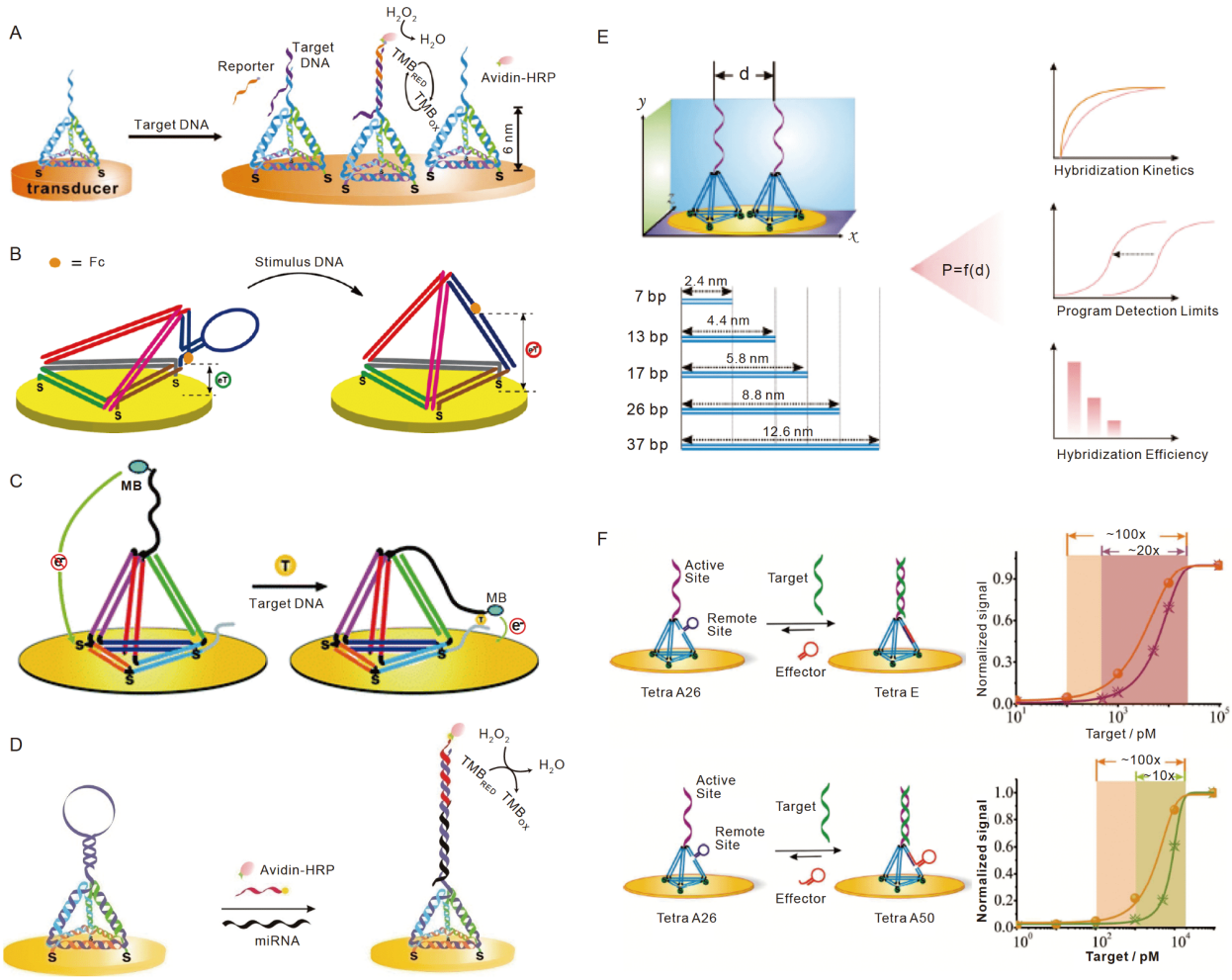

Figure 1 FNA-based electrochemical biosensors for nucleic acids analysis. Schematic representation of FNA-based (A) "sandwich-type" (Pei et al., 2010), (B) "nanoswitching-type" (Abi et al., 2014), (C) "swing-type" (Li et al., 2018a), and (D) "molecular beacon-type" (Lin et al., 2014) E-DNA biosensors. E, FNA-based E-DNA biosensors with different sizes of FNAs (Lin et al., 2015). F, FNA-based E-DNA biosensors with allosteric FNAs (Song et al., 2016).

Nucleic acid analysis with amplification process

In order to analyze the low abundance of target molecules, various application strategies, including multi-labeling, hybridization chain reaction (HCR) amplification, catalyzed hairpin assembly (CHA) amplification, catalytic recycling amplification, and rolling circle amplification, have been developed to enhance the sensitivity of FNA-based E-DNA biosensors (Lin and Zuo, 2018).

Multi-labeling strategy

The multiple signal system is an effective signal amplification strategy without the need for further amplification reaction. As shown in Figure 2A, Wen et al. reported an ultrasensitive electrochemical miRNA biosensor (EMRS), complemented with poly-HRP80, a polymerized streptavidin-HRP conjugate with up to 400 HRP molecules on each conjugate. Using this biosensor, the LOD of miR-21 reached 10 amol L^{-1}. The background noise produced by the polymerized streptavidin-HRP was comparable to that of the traditional HRP group (Wen et al., 2012; Wen et al., 2013).

Similar to the EMRS strategy, Xu et al. constructed a mecA DNA biosensor, employing a DNA tetrahedron-structure capture probe (TSCP) and seven biotin-labeled signal probes, which is called a multi-signal probe (MSP) system (Figure 2B). In this work, MSP played two important roles, i.e., amplifying signals with seven biotin molecules, and improving the accessibility of capture probes through target sequences. The biosensor has an LOD of 10 fmol L^{-1} for synthetic target DNA, which is three magnitudes lower than a single signal probe system (Xu et al., 2018). Huang et al. reported on an FNA-based E-DNA biosensor for miRNA, using guanine nanowire amplification, which can self-assemble numerous G-quadruplexes. This biosensor had an LOD of 176 fmol L^{-1}, with a linear operating range of 500 fmol L^{-1} to 10 nmol L^{-1} (Huang et al., 2017).

Zeng et al. reported a novel E-DNA biosensor, which

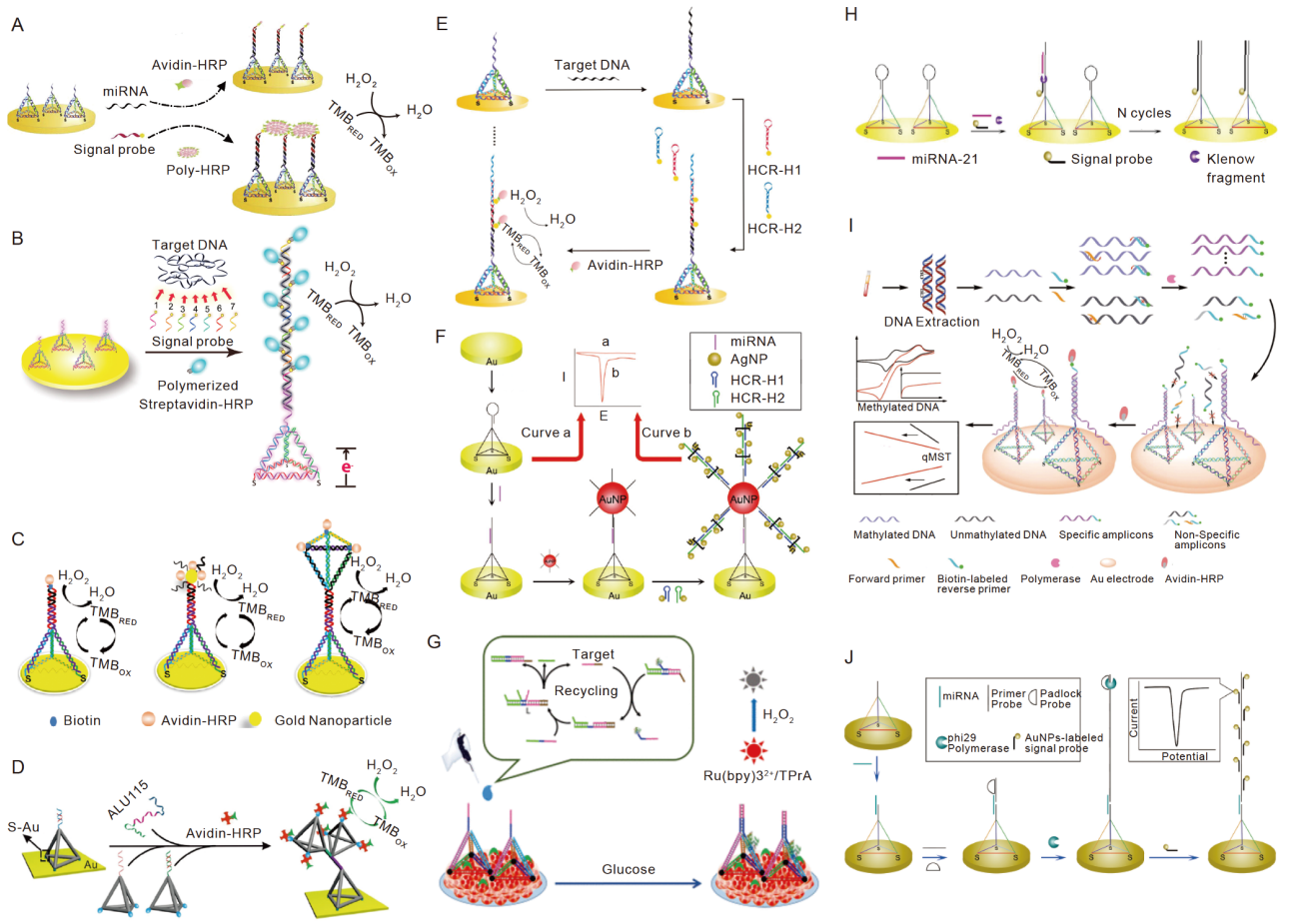

Figure 2 FNA-based electrochemical biosensors for nucleic acid analysis with amplification strategies. A–D, Multi-labeling strategy for signal amplification (Liu et al., 2018b; Wen et al., 2012; Xu et al., 2018; Zeng et al., 2015). E and F, HCR strategy for signal amplification (Ge et al., 2014; Miao et al., 2015a). G, CHA strategy for signal amplification (Feng et al., 2017). H and I, Catalytic recycling strategy for signal amplification (Miao et al., 2015b; Wang et al., 2017c). J, Rolling circle amplification strategy for signal amplification (Miao et al., 2015c).

consisted of two TDNs for sensitive detection of target DNA (Figure 2C). In this system, thiol- and biotin-modified DNA tetrahedral nanostructures were used as capture and reporter probes, respectively. The biotin-tagged TDNs were used to amplify the signal by capturing multiple catalytic enzymes. Such E-DNA biosensors can sensitively detect 1 fmol L^{-1} DNA targets (Zeng et al., 2015).

Liu et al. implemented an ultrasensitive sensing platform, in which FNAs served as valence-controlled signal amplifiers with high modularity (Figure 2D). This sensing platform enables the detection of tumor-relevant circulating free DNA (cfDNA), which is enhanced by three to five orders of magnitude, when compared with ssDNA functional electrodes (Liu et al., 2018b). They also adjusted the dynamic range of the biosensor by controlling the valence of the signal.

Hybridization chain reaction amplification strategy without enzymes

HCR is a novel amplification strategy based on cascade hybridization reactions triggered by initiators or target molecules (Choi et al., 2010; Dirks and Pierce, 2004; Evanko, 2004; Huang et al., 2013; Li et al., 2017).

As an example, Ge et al. reported an miRNA detection strategy by combining the FNA-based probes and HCR amplification (Figure 2E). The LODs for DNA and miRNA were 100 and 10 amol L^{-1}, respectively, which was three orders of magnitude higher than other "sandwich-type" E-DNA biosensors. Notably, 3D FNAs can improve the efficiency of signal amplification by capturing multiple catalytic enzymes (Ge et al., 2014). Miao et al. reported an miRNA sensor, which uses multiprobes assembled on gold nanoparticles (AuNPs) and dual amplification of HCR (Figure 2F), with a low LOD of 2 amol L^{-1} (Miao et al., 2015a).

Liu et al. constructed an electrochemical biosensor for miRNA-21 detection by fabricating TND probes on electrodes, which were prepared using a layer-by-layer assembly of oxidized single-walled carbon nanotubes and nanodiamonds. They achieved an ultrasensitive analysis with an

LOD of 1.95 fmol L^{-1}, by combining HCR with DNA-functionalized AuNPs, which serve as amplification enzymes (Liu et al., 2015a). Chen et al. developed an FNA-based biosensor for DNA methylation detection by employing a combined amplification strategy involving HCA, enzymatic catalysis, and AuNPs assembly. It had an LOD of ~0.93 amol L^{-1} with an operating range of 1 amol L^{-1} to 1 pmol L^{-1} (Chen et al., 2019b).

Catalyzed hairpin assembly amplification strategy without enzymes

CHA is a widely used amplification strategy, which is based on a recycling strand displacement reaction, triggered by initiators or target molecules (Karunanayake Mudiyanselage et al., 2018; Turberfield et al., 2003; Yin et al., 2008).

For example, Feng et al. reported an FNA-based electrochemiluminescence (ECL) biosensor using a CHA strategy (Figure 2G). In this work, programmable DNA cyclic amplification was activated and a glucose oxidase-DNA sequence (GOD-S) was released via toehold-mediated strand displacement in the presence of target DNA. As a result, the LOD of the ECL biosensor reached 40 amol L^{-1} (Feng et al., 2017).

Feng et al. constructed a novel, FNA-based, ECL biosensor using *in situ* surface-confined DNA assembly and amplification. The target DNA opened the H1 structures, while the products were used to open the H2 structures, releasing target DNA for the next recycling. This biosensor achieved an LOD of 20 amol L^{-1} with a linear operating range of 50 amol L^{-1} to 10 pmol L^{-1} (Feng et al., 2018).

Catalytic recycling amplification strategy with enzymes

Enzymes have also been used in amplification strategies, due to their novel activity (Weizmann et al., 2006; Xue et al., 2015; Zuo et al., 2010). For example, Miao et al. developed an FNA-based biosensor for miRNA detection, using strand displacement polymerization with Klenow fragments (Figure 2H). Taking advantage of strand displacement polymerization, more silver nanoparticles (AgNPs) were fabricated on the electrode surface in the presence of target miRNA. This biosensor was highly sensitive, with an LOD of 0.4 fmol L^{-1} (Miao et al., 2015b).

Wang et al. reported a FNA-based biosensor for circulating methylated DNA using a sequential discrimination–amplification strategy (Figure 2I). Based on dual sequence discrimination and cascade signal amplification reaction, this biosensor exhibited a high sensitivity to methylated DNA, capable of identifying one copy in a 1,000-fold excess of unmethylated alleles (Wang et al., 2017c).

Rolling circle amplification (RCA) strategy with enzymes

RCA is a typical isothermal amplification strategy based on enzymes, which has been applied in the construction of numerous biosensors (Larsson et al., 2004; Liu et al., 2017; Liu et al., 2015b; Zhao et al., 2008; Zhu et al., 2013). For example, Liu et al. developed an ultrasensitive FNA-based E-DNA biosensor for DNA methylation detection using RCA strategy. Due to the high DNAzyme activity of the RCA products, the LOD of this biosensor reached 0.1 fmol L^{-1} for the target methylated DNA with a working detection range of 10^{-15} to 10^{-9} mol L^{-1} (Liu et al., 2018a).

With a similar approach, Miao et al. constructed an ultrasensitive analysis platform for miRNA detection (Figure 2J). The FNA-based probes on the electrode surface were used to recognize the target miRNA, while primer probes triggered RCA on the electrode surface. The ssDNA functionalized silver nanoparticles (AgNPs) hybridized with the RCA products, which produced amplified electrochemical signals. The LOD of this biosensor reached 50 amol L^{-1} (Miao et al., 2015c).

FNA-based electrochemical biosensors for ions and small molecule analysis

Ion detection

The rapid, accurate, and convenient detection of heavy metal is of great importance to public health (Leung et al., 2019; Thekkan et al., 2019; Veetil et al., 2017; Xing et al., 2019). FNA-based electrochemical biosensors have played an important role in ion detection.

Bu et al. constructed a "turn-on" biosensor for Hg^{2+}, combining tetrahedron-structured DNA with a functionalized oligonucleotide (Figure 3A). In this system, Hg^{2+} nucleic acid probes were immobilized on the gold electrode surface with the assistance of FNAs. The LOD of this biosensor reached 100 pmol L^{-1}, which was two magnitudes lower than the ssDNA-based strategy control group (Bu et al., 2011).

Guo et al. reported a Pb^{2+} biosensor using FNAs and the DNAzyme against Pb^{2+} (Figure 3B). The DNAzyme sequences were inserted into the edge of the FNA, which was cleaved in the presence of Pb^{2+} to induce a change in the electrochemical signal. The LOD reached 0.01 μmol L^{-1}, with a detection range of 0.01 to 100 μmol L^{-1} (Guo et al., 2019).

Small molecule detection

FNA-based electrochemical biosensors can also be harnessed to detect small molecules, such as cocaine, adenosine triphosphate (ATP), dihydronicotinamide adenine dinucleotide (NADH), and 8-hydroxy-20-deoxyguanosine (8-OHdG)

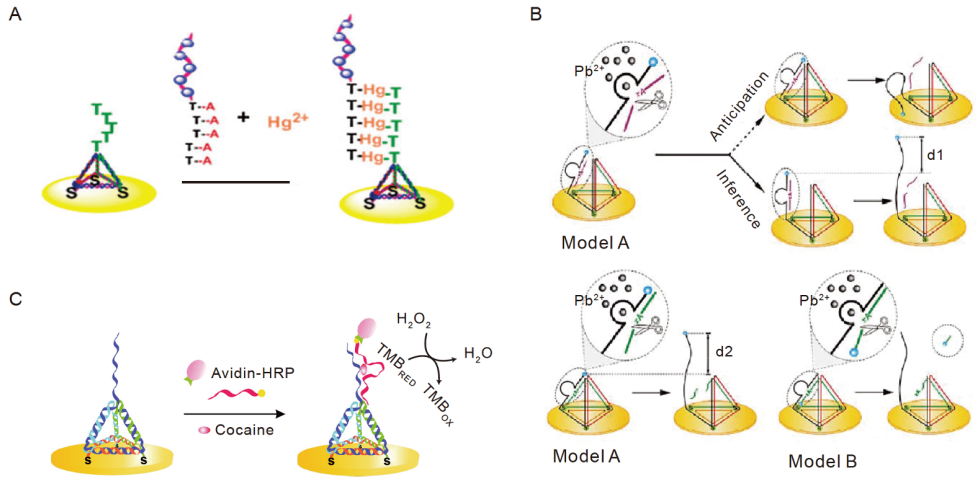

Figure 3 FNA-based electrochemical biosensors for ions and small molecules analysis. A and B, Ions analysis (Bu et al., 2011; Guo et al., 2019). C, Small molecules analysis (Wen et al., 2011).

(Bu et al., 2013; Fan et al., 2016; Li et al., 2015b; Wen et al., 2011).

Wen et al. constructed an ultrasensitive cocaine biosensor by implementing a cocaine aptamer with FNAs (Figure 3C). FNA-based probes with a pendent aptamer sequence were fabricated on the electrode surface. In the presence of cocaine, the split cocaine aptamer would fuse together with the assistance of the FNAs, inducing an electrochemical signal. The LOD of this biosensor reached 33 nmol L^{-1} (Wen et al., 2011). Similarly, Sheng et al. fabricated a cocaine sensor based on the structural transformation of FNAs. In the presence of cocaine, the structure of the aptamer-composed FNAs changed, which consequently induced an electrochemical signal. The LOD achieved 0.21 nmol L^{-1}, with a detection range of 1.0 nmol L^{-1} to 2.0 μmol L^{-1} (Sheng et al., 2014).

Bu et al. reported an ECL aptabiosensor for ATP, based on functionalized oligonucleotides. In this work, the target molecule, ATP, would replace the aptamer from the duplex structure, inducing the release of $Ru(phen)_3^{2+}$ in the intermolecular duplex. This analysis platform had an LOD of 0.2 nmol L^{-1} (Bu et al., 2013).

Li et al. constructed an ultrasensitive NADH analysis platform using gold electrodes modified with a graphene-DNA tetrahedron-AuNPs complex. The presented platform had an LOD of 1 fmol L^{-1}, with a detection range of 1 fmol L^{-1} to 10 pmol L^{-1} (Li et al., 2015b). Fan et al. reported an 8-OHdG sensor based on 8-OHdG aptamers and FNAs. In the presence of 8-OHdG and hemin, the 8-OHdG aptamer folded into a G-quadruplex structure, which triggered a polyaniline (PANI) deposition. This analysis platform exhibited highly sensitive detection of 8-OHdG, with an LOD of 1 pmol L^{-1} and a detection range of 10 pmol L^{-1} to 2 nmol L^{-1}. The sensitivity was enhanced almost 300-fold, when compared with the electrochemical biosensors previously reported (Fan et al., 2016).

FNA-based electrochemical biosensors for protein analysis

Protein analysis based on recognition of aptamer

Protein biomarkers are effective candidate markers for specific clinical objectives. FNA-based electrochemical biosensors functionalized with aptamers against specific proteins are widely used to detect specific target proteins (Zhou and Rossi, 2017).

Pei et al. constructed an FNA-based analysis platform with aptamer probes for sensitive detection of thrombin, a potential tumor marker (Figure 4A). This analysis platform showed an LOD of 100 pmol L^{-1}, which was 1,000 times less than ssDNA aptamer-based biosensors (Pei et al., 2010).

Sheng et al. reported an IFN-γ biosensing platform based on the structural conversion of FNAs and anti-IFN-γ aptamers (Figure 4B). Target molecules and corresponding aptamers were used to control the state of the FNAs and induce an electrochemical signal. The biosensor exhibited a highly sensitive detection of IFN-γ, with an LOD of 5.2×10^{-10} mol L^{-1}, and a linear detection range of 1.0×10^{-9} to 2.0×10^{-6} mol L^{-1} (Sheng et al., 2013).

Chen et al. developed an electrochemical biosensor for human epidermal growth factor receptor 2 (HER2), based on gold nanorod@Pd super-structures-aptamer-horseradish peroxidase (GNR@Pd SSs-Apt-HRP) complex (Figure 4C). In this work, the target molecule, HER2, was recognized and captured by its aptamer, and a sandwich-type structure of DNA tetrahedron-HER2-nanoprobes was constructed on the surface of the gold electrode to detect HER2. The LOD of this biosensor reached 0.15 ng mL^{-1} (Chen et al., 2019a).

Protein analysis based on antibody recognition

FNA-based electrochemical biosensors with antibodies are

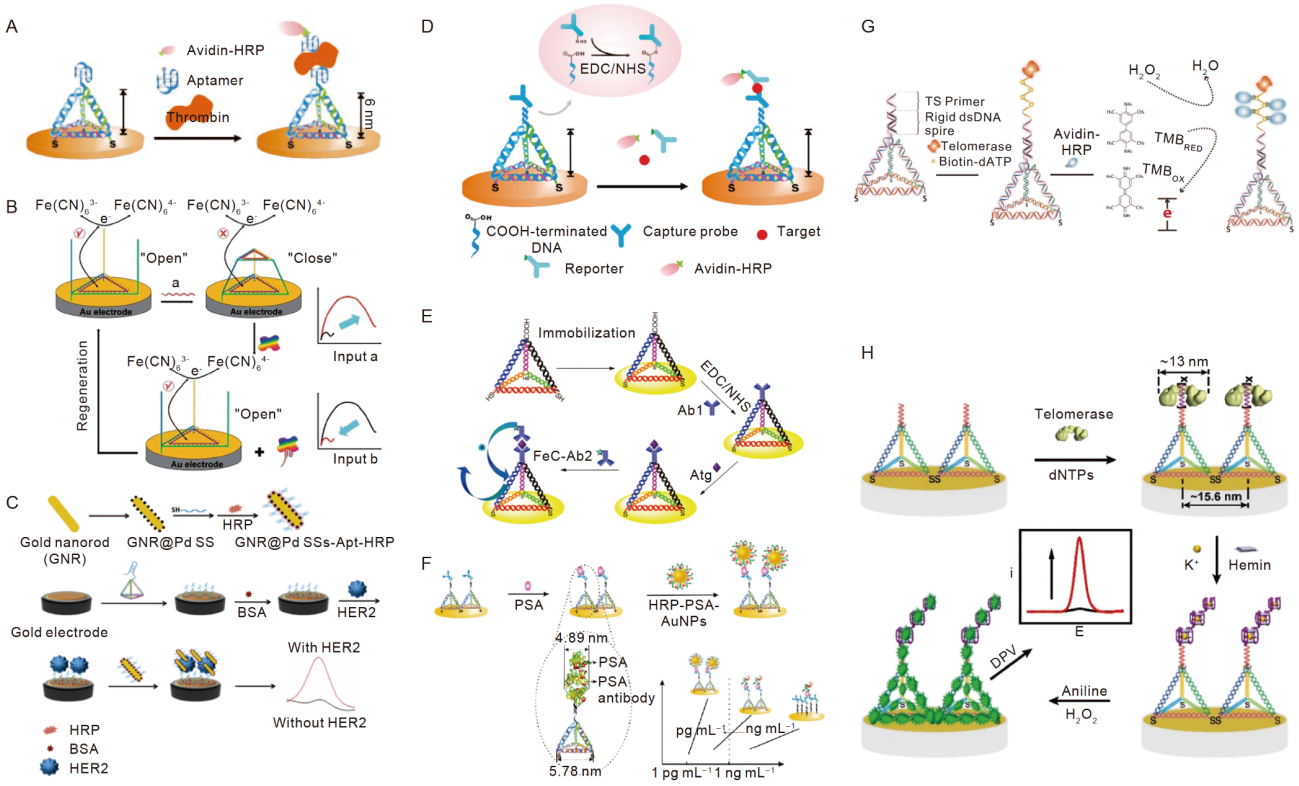

Figure 4 FNA-based electrochemical biosensors for protein analysis. Protein analysis based on (A–C) recognition of aptamer (Chen et al., 2019a; Pei et al., 2010; Sheng et al., 2013), (D–F) recognition of antibody (Chen et al., 2014; Pei et al., 2011; Yuan et al., 2014), and (G and H) specific activity of target protein (Li et al., 2015a; Liu et al., 2016).

also used to detect specific target proteins (Chen et al., 2014; Pei et al., 2011; Yuan et al., 2014).

For example, Pei et al. reported an electrochemical immunobiosensor for tumor-necrosis-factor alpha (TNF-α) (Figure 4D). This had a detection range of 100 pg mL^{-1} to 5 ng mL^{-1}(Pei et al., 2011).

Yuan et al. constructed an electrochemical sensing platform for immunoglobulin G (IgG) with antibody-functionalized FNAs (Figure 4E). The results demonstrated that FNAs with a hollow structure could facilitate electron transfer, which increases the sensitivity of electrochemical detection. The LOD of this sensing platform reached 2.8 pg mL^{-1} (Yuan et al., 2014).

Chen et al. developed an electrochemical immunobiosensor for prostate-specific antigen (PSA), by precisely assembling antibodies on the electrodes with FNAs (Figure 4F). In this work, by optimizing the nanoscale-spacing of immobilized antibodies and amplifying the signal with AuNPs, extremely sensitive detection of PSA was achieved, with an LOD of 1 pg mL^{-1} (Chen et al., 2014).

Protein analysis based on the specific activity of target protein

For telomerase analysis, its reverse transcription activity is used to report signals. For example, Li et al. reported a telomerase biosensor based on a telomere strand primer (TSP) pendent of a DNA tetrahedron (Figure 4G). By precisely controlling the distance between TSPs on the electrodes with FNAs, they achieved higher signal gains, when compared with the traditional TSP system without FNAs. The LOD reached 10 HeLa cells (Li et al., 2015a).

Liu et al. reported a label-free telomerase biosensor by using telomeric hemin/G-quadruplex triggered polyaniline deposition on the DNA tetrahedron-structure platform (Figure 4H). By optimizing the FNA size, they enhanced telomerase accessibility, reactivity, and detection sensitivity. The LOD reached 1 HeLa cell, with a dynamic range of 5 to 5,000 HeLa cells (Liu et al., 2016).

Feng et al. developed a surface plasmon resonance (SPR) enhanced ECL biosensor for telomerase. The distance between the SPR nanoparticles was accurately controlled, which enhanced the SRP-ECL coupling effect. The LOD reached 2.03×10^{-9} IU for CdS QDs and 1.45×10^{-9} IU for luminol (Feng et al., 2017).

FNA-based electrochemical biosensors for cellular and exosome analysis

Cancer cell analysis

The sensitive detection of cancer cells plays a critically im-

portant role in the early detection of cancer and cancer metastasis (Deng et al., 2017; Li et al., 2014; Zhu et al., 2017). FNA-based electrochemical biosensors can reveal sensitive detection of cancer cells (Chen et al., 2018; Zhou et al., 2014).

Zhou et al. reported an ultrasensitive biosensor for cancer cells by using FNAs and an HCR amplification strategy (Figure 5A). In this work, they achieved the detection sensitivity of four cancer cells (Zhou et al., 2014). Chen et al. constructed an FNA-based electrochemical aptabiosensor for human liver hepatocellular carcinoma cells (HepG2), using a multibranched HCR amplification strategy. With the assistance of aptamer-functionalized FNA probes on the electrodes, HepG2 cells were recognized and captured on the electrode surface. The LOD of this established biosensor was 5 cells per mL with a broad detection range of 10^2 to 10^7 cells per mL (Chen et al., 2018).

Exosomes analysis

Exosomes (extracellular vesicles, 50–100 nm) circulating in biofluids as intercellular signal transmitters, have been reported as tumor biomarkers. The development of exosome detection is important for cancer diagnosis (Skog et al., 2008; Valadi et al., 2007). Giovanni et al. developed an FNA-assisted aptabiosensor for hepatocellular exosomes, combining aptamer technology, FNA, and portable electrochemical devices, as shown in Figure 5B. Due to oriented immobilization, aptamers significantly improved the accessibility for suspended exosomes, and the FNA-assisted aptabiosensor could detect exosomes with two orders of magnitude higher sensitivity, when compared with the single-stranded aptamer-functionalized aptabiosensor (Wang et al., 2017b).

Bacteria analysis

Effective bacteria detection, such as pathogen detection, is necessary for the treatment of infectious diseases (Shen et al., 2016; Tram et al., 2014). FNA-based electrochemical biosensors have been used in bacteria detection with high sensitivity (Giovanni et al., 2015; Wang et al., 2017a).

Giovanni et al. reported an FNA-based immunological biosensor for *E. coli* bacteria using the antibodies against the *E. coli* bacteria, as shown in Figure 5C. Based on the "sandwich-type" strategy, the biosensor achieved sensitive detection of *E. coli* lipopolysaccharides, with an LOD of 0.20 ng mL^{-1} *E. coli* lipopolysaccharides and 1.20 CFU mL^{-1} equivalent of lysed *E. coli* bacteria (Giovanni et al., 2015).

Wang et al. constructed an electrochemical immunobiosensor for isolating pneumococcal surface protein A (PspA) peptides and *Streptococcus pneumoniae* (SP) lysates from synthetic and actual human samples (Figure 5D). This FNA-based immunobiosensor exhibited excellent electrochemical sensing ability toward PspA, with an LOD of

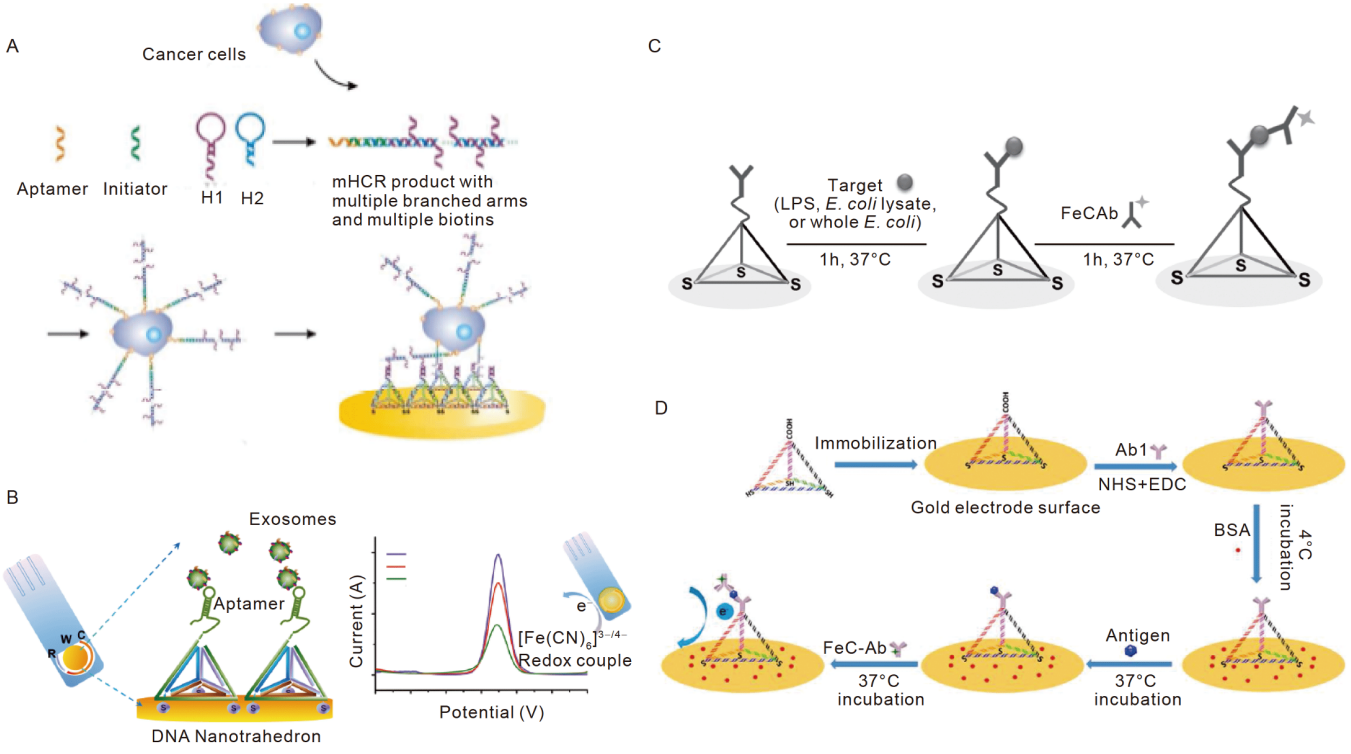

Figure 5 FNA-based electrochemical biosensors for cells and exosomes analysis. A, Cancer cell analysis (Zhou et al., 2014). B, Exosomes analysis (Wang et al., 2017b). C and D, Bacteria analysis (Giovanni et al., 2015; Wang et al., 2017a).

0.218 ng mL^{-1} and a linear range of 0 to 8 ng mL^{-1}. In addition, this immunobiosensor had a good sensing performance toward SP lysates, with an LOD of 0.093 CFU mL^{-1} and a linear range of 5 to 100 CFU mL^{-1} (Wang et al., 2017a).

Conclusion and outlook

With the ability to immobilize biomolecules in a highly controlled orientation, coupled with the programmable density control functions (Deußner-Helfmann et al., 2018; Strauss et al., 2018), FNAs are appealing for a broad range of electrochemical biosensor designs. In this review, we have summarized the application of FNA-based electrochemical biosensors in the detection of different targets, including nucleic acids, ions, small molecules, proteins, and cells.

The FNAs have greatly promoted the development of biosensors, especially for electrochemical biosensors. There are still challenges for FNA-based electrochemical biosensors to be able to satisfy different analysis requirements. For example, analysis with FNA-based electrochemical biosensors at the single-molecule and/or single-cell level remains a challenge, especially for non-invasive analyses. Achieving the ultrasensitive analysis of specific target molecules at the single-cell level will lead to breakthroughs in clinical and biological applications. In addition, multiplex detection should be further explored, which can be achieved by combining multi-type probes on more robust biosensor interfaces. FNAs can provide enormous potential for constructing multi-probe platforms on electronic interfaces. The application of FNA-based ultrasensitive biosensors in different scenarios, such as intracellular or *in vivo*, should also be expanded. The precision of DNA nanotechnology provides a quantitative approach to discovering the underlying mechanisms of biomolecular interfaces. This understanding increases our ability to manipulate the biosensing interfaces and create better biosensors, and can be used generally to develop a variety of biomolecular devices for electrochemical analysis.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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