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Review

Recent Progress in Electrochemical Aptasensors: Construction and Application

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Abstract: Electrochemical aptasensors have gained significant attention due to their exceptional sensitivity, selectivity, stability, and rapid response, combining the advantages of electrochemical techniques with the specific recognition ability of aptamers. This review aims to provide a comprehensive summary of the recent advances in electrochemical aptasensors. Firstly, the construction method and the advantages of electrochemical aptasensors are introduced. Subsequently, the review highlights the application progress of electrochemical aptasensors in detecting various chemical and biological molecules, including metal ions, small biological molecules, drugs, proteins, exosomes, tumor cells, bacteria, and viruses. Lastly, the prospects and challenges associated with electrochemical aptasensors are discussed.

Keywords: electrochemical aptasensor; aptamer; biochemical analysis; disease diagnosis

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1. Introduction

Electrochemical biosensors have been widely applied in many fields including environmental monitoring, food safety, disease diagnosis, agricultural engineering, and even public safety owing to their excellent advantages of high sensitivity, fast analysis speed, simple operation, high reproducibility, long-term stability, ease of miniaturization, and on-site/in situ analysis [1–3]. Nowadays, antibody-based bio-molecular recognition events are the general way to construct electrochemical biosensors for the recognition and detection of target molecules. For example, different electrochemical immunosensors have been developed for the analysis of SARS-CoV-2 [4], cardiac troponin I [5], carcinoembryonic antigen [6], etc. Though great advances in electrochemical immunosensors have been achieved, they still have some limitations in the application of environmental monitoring, small biological molecules, and drug metabolism analysis due to the specific recognition scope of antibodies. Therefore, it is necessary to develop other electrochemical biosensors to enable sensitive and selective detection of target molecules.

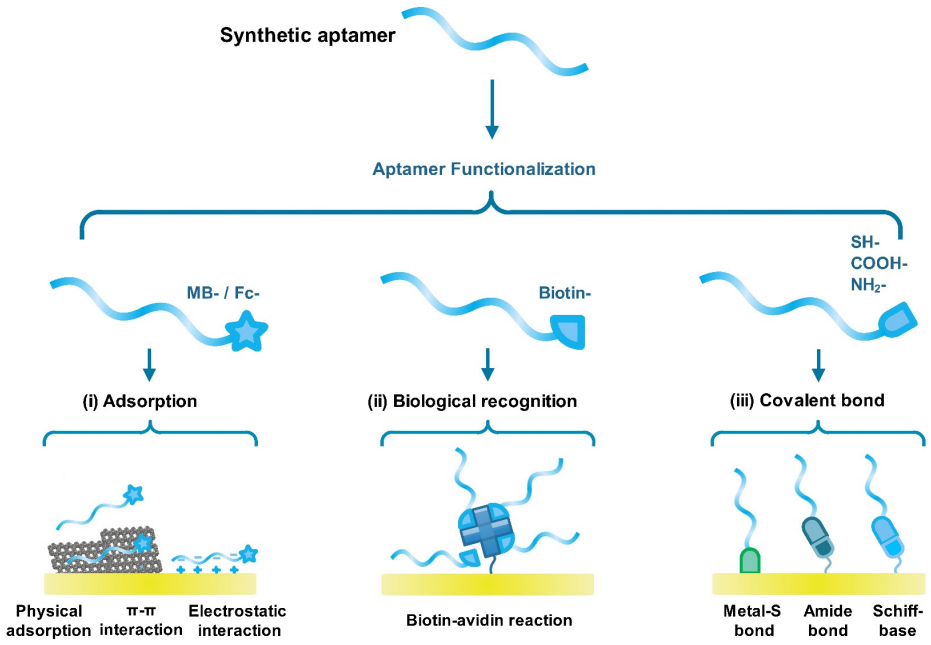
In general, an aptamer is a single-stranded DNA or RNA sequence, which is selected by systematic evolution of ligands by exponential enrichment (SELEX) in vitro, which utilizes protein-, cell-, and live animal-based selection processes [7]. Like antibodies, aptamers have high specificity and affinity for a wide range of target substances, including metal ions, biological small molecules, proteins, exosomes, cells, and microbial pathogens [8–10]. Therefore, it is considered as a promising alternative bio-molecular recognition element. More importantly, the unique nature of aptamers including easy synthesis, multiplexed functionalization, and higher stability than antibodies make them widely applicable in the field of analytical chemistry. Coupled with the electrochemical technique, the aptamer-based electrochemical biosensor (also called electrochemical aptasensor) has gradually become a research hotspot due to its excellent performance and wide application fields [11–14]. With the fast development of nanotechnology, the introduction of nanomaterials greatly improves the analytical performance of electrochemical aptasensors [15–17]. For example, Ahmadi and co-workers have

constructed an electrochemical aptasensor based on gold nanoparticles (AuNPs)/nitrogen-doped carbon nano-onions (NCNOs) for the detection of *Staphylococcus aureus* (*S. aureus*) [18]. The synergetic effect of AuNPs and NCNOs effectively enhanced the active surface area and the conductivity of the electrode, leading to an outstanding performance of the designed aptasensor with a wide linear range (10–10^8 CFU/mL) and a low detection limit (3 CFU/mL). This biosensor also showed excellent repeatability, reproducibility, and long-term stability, allowing it to detect low levels of *S. aureus* in human serum samples. Additionally, to improve the sensitivity and feasibility of the photoelectrochemical (PEC) aptasensor, Ju et al. decorated $MgIn_2S_4$ nanoplates on a TiO_2 nanowire array (TiONA) to form $MgIn_2S_4$-TiONA heterojunctions for the construction of a visible light PEC aptasensing platform [19]. The designed PEC aptasensor showed excellent performance for adenosine triphosphate (ATP) detection due to the large surface area and strong absorption efficiency of the $MgIn_2S_4$-TiONA heterojunction. The outstanding detection performance has greatly promoted the development of nanomaterial-based electrochemical aptasensors in the analytical fields.

In recent years, great advances in electrochemical aptasensors have been achieved. In this review, we are aiming to give a comprehensive overview of the recent progresses in electrochemical aptasensors with some typical examples: (1) to summarize the immobilization methods of the aptamer as the key recognition unit; (2) to cover a variety of target substances for detection applications in different fields; (3) to put insightful comments on various detection strategies with high sensitivity, specificity, and selectivity and give the exploration experiences and underlying experimental regularity. Finally, the opportunities and challenges of electrochemical aptasensors in the future are also discussed, which will be beneficial to clinical applications or commercial transformations of scientific research.

2. Immobilizations of Aptamers on the Surface of Electrode

How to efficiently immobilize aptamers on the electrode surface is a key step in the construction of electrochemical aptasensors, which greatly affects the sensing performance and application fields [20,21]. In general, electrochemical indicator- (such as methylene blue, ferrocene), thiol-, biotin-, and carboxyl-labeled aptamers have been synthesized by companies, which are designed for sensing purposes. Nowadays, three methods including adsorption, biological recognition, and covalent bonds have been widely used to construct electrochemical aptasensors (Scheme 1). The advantages and disadvantages of these immobilization methods are listed in Table 1 [22–24].



Scheme 1. A general scheme of the modification and immobilization of aptamers on sensing interfaces.

Table 1. Immobilization methods for the construction of electrochemical aptasensors.

Immobilization Methods	Technique Principles	Advantages	Disadvantages	References
adsorption method	electrostatic interaction, physical adsorption, π–π interaction	no chemical modification, simple, fast	easy detachment, low density, poor orientation	[25–27]
biological recognition	biotin–avidin reaction	mild conditions, high immobilization efficiency, orderly assembly	decrease in electrochemical signal	[28–30]
covalent bond	metal-S bond, amide bond, Schiff-base	good stability, high recognition activity, easy regeneration, adjustable process	complex chemical modification, introduced interferences	[31–33]

2.1. Adsorption Method

As a nucleic acid sequence, aptamers usually have negative charges due to their phosphate backbone. Therefore, aptamers easily adsorb on the surface of nanomaterials with positive charges via electrostatic reaction [10,34], such as metal–organic frameworks (MOFs) [35], mesoporous silica nanoparticles (MSNs) [36], and magnetic nanoparticles (MNPs) [37]. Moreover, aptamers also easily adsorb on the modified electrode surface or nanomaterials' surface through physical adsorption or π–π interaction, such as graphene [38], carbon nanotubes [39], single-layered MoS_2 [40], noble metal nanoparticles [41], etc. This immobilization method is simple, fast, and without chemical modification. However, this method has obvious shortcomings including easy detachments (sensitivity to pH, ionic strength, temperature, etc.), low density, and poor orientation on the sensing interfaces [26,27].

2.2. Biological Recognition

The biological recognition system has been recently employed to immobilize aptamers on the electrode surface and nanomaterials' surface through affinity labeling strategies, such as the biotin–streptavidin recognition reaction [42]. Generally, one streptavidin molecule has four identical subunits, which can specifically bind up to four biotin molecules. The binding constant (Ka) of biotin–streptavidin is about 10^{15} mol^{-1}, suggesting that this biological recognition is specific and efficient [43]. However, the poor conductivity of biotin and streptavidin molecules often leads to a decrease in the signal of electrochemical aptasensors, resulting in low detection sensitivity [28–30].

2.3. Covalent Bond

The covalent bond is a popular method for the construction of electrochemical aptasensors. To achieve this purpose, it usually requires the synthesized aptamer with specific functional groups such as thiol, amino, carboxylic acid, or phosphate groups [44,45]. Correspondingly, noble metal nanoparticles or various active groups including hydroxyl and carboxyl are introduced to the electrode surface. Through a chemical reaction, an aptamer can be efficiently and controllably immobilized on the electrode surface. This method for the construction of electrochemicals exhibits several advantages of good stability, high binding efficiency, easy regeneration, and a controllable process [31–33]. Meanwhile, this method required chemical modification, complicated steps, and introduced other interference factors [46,47].

Besides immobilization methods, experimental conditions are important factors to greatly affect the detection performance of electrochemical aptasensors, such as pH value, aptamer concentration, binding time between aptamer and target molecules, elution time, and temperature [48–50]. It should be noted that the experimental conditions are generally optimized according to the designed detection strategies and the properties of introduced nanomaterials and target molecules. Taking the pH value effect as an example, Liu et al. found that the electrochemical signal of the designed aptasensor reached a maximum

value when the pH value was 7.0. Low and high pH could affect the binding efficiency of the aptamer and dopamine [51]. In another work, the optimized pH value of the developed aptasensor for Aflatoxin B1 detection was about 4.0, which is greatly dependent on the properties of the introduced titanium carbide/carboxylated graphene oxide-poly(4-vinyl pyridine) nanocomposites [52]. For the temperature effect, many electrochemical aptasensors are typically used at room temperature. Especially, electrochemical aptasensors for biological molecules' detection are usually used at 37 degrees Celsius, the optimized temperature, which is close to normal human body temperature [53,54]. Therefore, suitable experimental conditions could result in better detection performance.

3. Application of Electrochemical Aptasensors for the Detection of Different Target Molecules

3.1. Detection of Metal Ions

The detection of metal ions is particularly important in both environmental monitoring and biomedical diagnosis [55,56]. In recent years, with the acceleration of industrialization, environmental pollutants have become an increasingly serious problem. Among them, heavy metal ions, as a kind of pollutant, are difficult to degrade under natural conditions and can be accumulated through the food chain to harm human health. Therefore, it is an urgent problem to develop rapid, simple, and efficient sensors for qualitative and quantitative analysis of heavy metal ions in the environment.

Although traditional methods (such as atomic absorption spectroscopy, inductively coupled plasma mass spectrometry, high-performance liquid chromatography, etc.) have achieved high detection performance, the high cost of instruments and the need for specialized operators have limited their widespread application. Compared to traditional analytical methods, electrochemical methods show many advantages for heavy metal ions' detection due to their real-time detection capability, high sensitivity, fast response time, miniaturization, and integration [57,58]. To further improve detection selectivity in complex environments, electrochemical aptasensors are considered as promising detection methods for the determination of heavy metal ions [1,59]. For example, Zhang et al. designed an electrochemical aptasensor for trace heavy metal ions' detection based on the Fe(III)-based metal–organic framework (Fe-MOF) and mesoporous Fe_3O_4@C nanocapsules core-shell nanocomposite (denoted as Fe-MOF@mFe_3O_4@mC) [60]. The designed nanocomposites showed excellent electrochemical performance and high loading of aptamers, resulting in the high performance of this aptasensor for Pb^{2+} and As^{3+} detection. Under optimal conditions, the constructed aptasensor can detect as low as 2.27 pM Pb^{2+} and 6.73 pM As^{3+}, respectively. Besides the selective determination of metal ions, Gao et al. developed a dual signal-based electrochemical aptasensor for the simultaneous detection of Pb^{2+} and Hg^{2+} in environmental water samples by in situ assembling electrochemical signal tags (Cu^{2+}-Melamine and Nile blue) on the terminal of Pb^{2+}-binding aptamer (PBA) and Hg^{2+}-binding aptamer (HBA), respectively (Figure 1A) [61]. Once specifically capturing target Pb^{2+} and Hg^{2+}, the two electrochemical signal tags are far away from the electrode surface due to the rigidity of PBA-Pb^{2+} and HBA- Hg^{2+} secondary structures, leading to electrochemical "signal-off" with the increase in Cu^{2+}-Melamine and Nile blue. Based on this detection mechanism, the detection limits of the developed aptasensor for Pb^{2+} and Hg^{2+} analysis are 0.98 pM and 19 pM, respectively. Moreover, the designed aptasensor could achieve the successful determination of Pb^{2+} and Hg^{2+} in real water samples, presenting its latent capacity in the monitoring of heavy metal ions.

Metal ion concentration in the body also plays an important physiological role in maintaining the balance of intracellular and extracellular fluids, as well as the excitability of nerves and muscles [62,63]. It has been found that abnormal metal ion concentration is closely related to a variety of nervous system diseases [64]. For example, copper ions (Cu^{2+}), zinc ions (Zn^{2+}), iron ions (Fe^{3+}), and aluminum ions (Al^{3+}) are involved in the pathogenesis of Alzheimer's disease (AD) [65]. Therefore, it is significant to monitor these metal ions' concentrations for the early diagnosis of AD patients. For the sensitive

and selective determination of Zn^{2+}, Zhao and co-workers designed an electrochemical aptasensor by fixing aptamers on the surface of bi-functional SiO_2-Pt@meso-SiO_2 core-shell nanoparticles [66]. The developed bi-functional nanoparticles can not only greatly enhance the conductivity of the electrode but also can pre-enrich Zn^{2+} onto the electrode surface. As a result, this aptasensor can determine the Zn^{2+} concentration as low as 65 pM. Moreover, this aptasensor can successfully detect Zn^{2+} in human blood and disrupted human cells. Similarly, Salehan and co-workers proposed an electrochemical aptasensor for potassium ion (K^+) analysis through electrochemical impedance spectroscopy [67]. They assembled the selected aptamer [$G_3(T_2AG_3)_3$] onto the surface of a polyaniline (PANI) modified electrode via electrostatic adsorption. In the presence of K^+, a single-strand aptamer folds into a G-quadruplex configuration, which blocks the electron transfer between the electrochemical indicator and electrode surface (Figure 1B). As a result, the charge transfer resistance (R_{ct}) increased with the increasing concentration of K^+. The linear relationship between ΔR_{ct} and the logarithm of K^+ concentration is plotted ranging from 10 pM to 60 μM with a detection limit of 3.7 pM. Moreover, this developed aptasensor can efficiently determine K^+ in serum samples, suggesting that it is a promising method for metal ion detection.

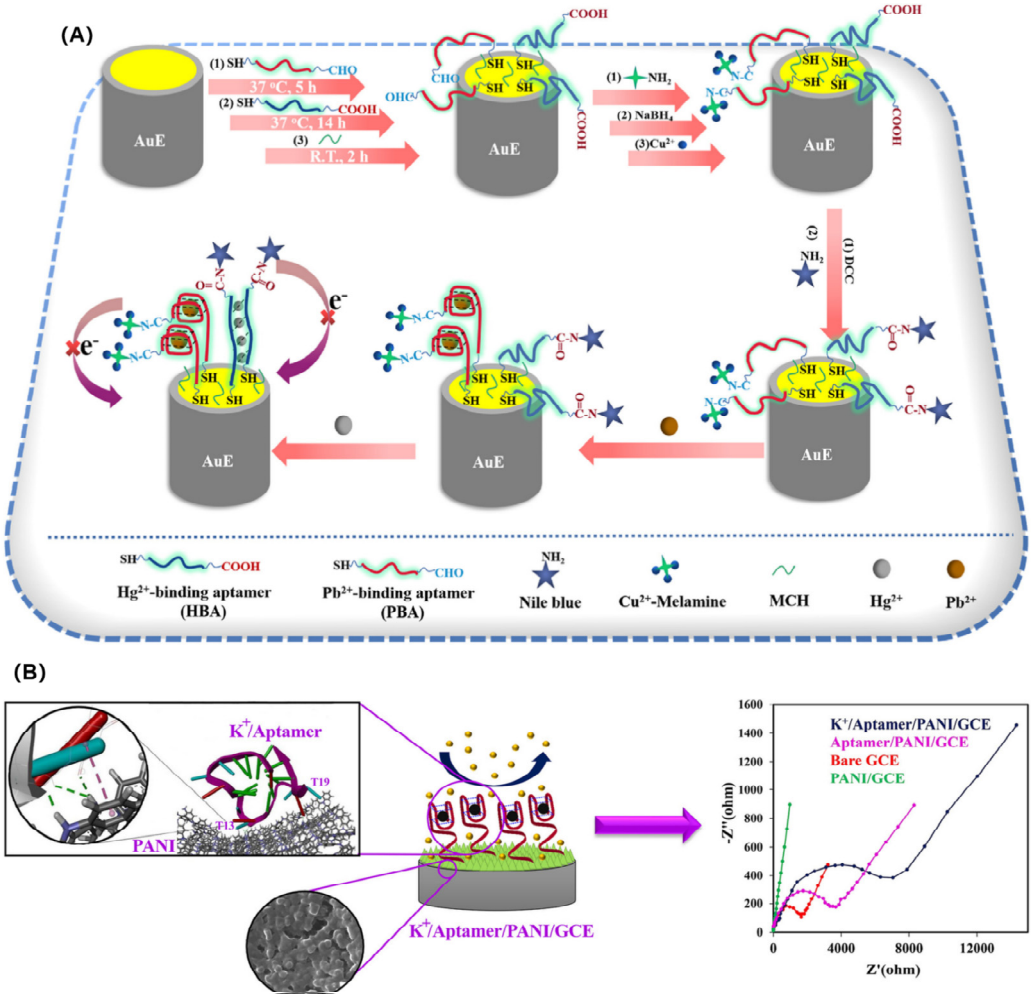


Figure 1. (**A**) Fabrication illustration of the dual signal−based electrochemical aptasensor for simultaneous analysis of Pb^{2+} and Hg^{2+}. (Reproduced with permission from Ref. [61] Copyright 2022, Elsevier). (**B**) Scheme of the impedimetric biosensor for the determination of potassium ions based on polyaniline/GCE and DNA G-quadruplex conformation. (Reproduced with permission from Ref. [67] Copyright 2022, Elsevier).

3.2. Detection of Pesticides

Over decades, the over-range and excessive dosage use of pesticides not only causes environmental pollution but also directly or indirectly threatens human health due to the long-term accumulation of residues. Nowadays, gas chromatography (GC), high-performance liquid chromatography (HPLC), GC-mass spectrometry (MS), and fluorescence and electrochemistry methods have been widely used to determine pesticides with accepted sensitivity, selectivity, and efficiency [68]. It is noted that some methods have disadvantages in the analysis of pesticides, such as low sensitivity, poor selectivity, and high cost [69]. Therefore, it is necessary to develop new methods for the sensitive and efficient detection of pesticides.

Electrochemical aptasensors have efficiently addressed these issues in pesticide detection. For example, Venegas and co-workers developed a label-free electrochemical aptasensor for the selective detection of carbendazim (CBZ) [70]. As shown in Figure 2A, the oxidized carbon nanotubes (CNT-COOH) were deposited on the surface of screen-printed carbon electrodes (SPCE) via physical adsorption. Then, amine-labeled aptamer ($AP\text{-}NH_2$) was assembled on the surface of SPCE via covalent immobilization. In the presence of CBZ, the corresponding aptamers specifically capture analytes to form secondary structures, causing a decrease in the electrochemical signal due to the blocking of electron transfer. According to this phenomenon, this proposed electrochemical aptasensor can detect values as low as 4.35 nM (0.83 ng/mL) CBZ, which is comparable to the HPLC method. Moreover, this aptasensor can efficiently determine CBZ in tomatoes, proving that the aptasensor can detect pesticides in food samples.

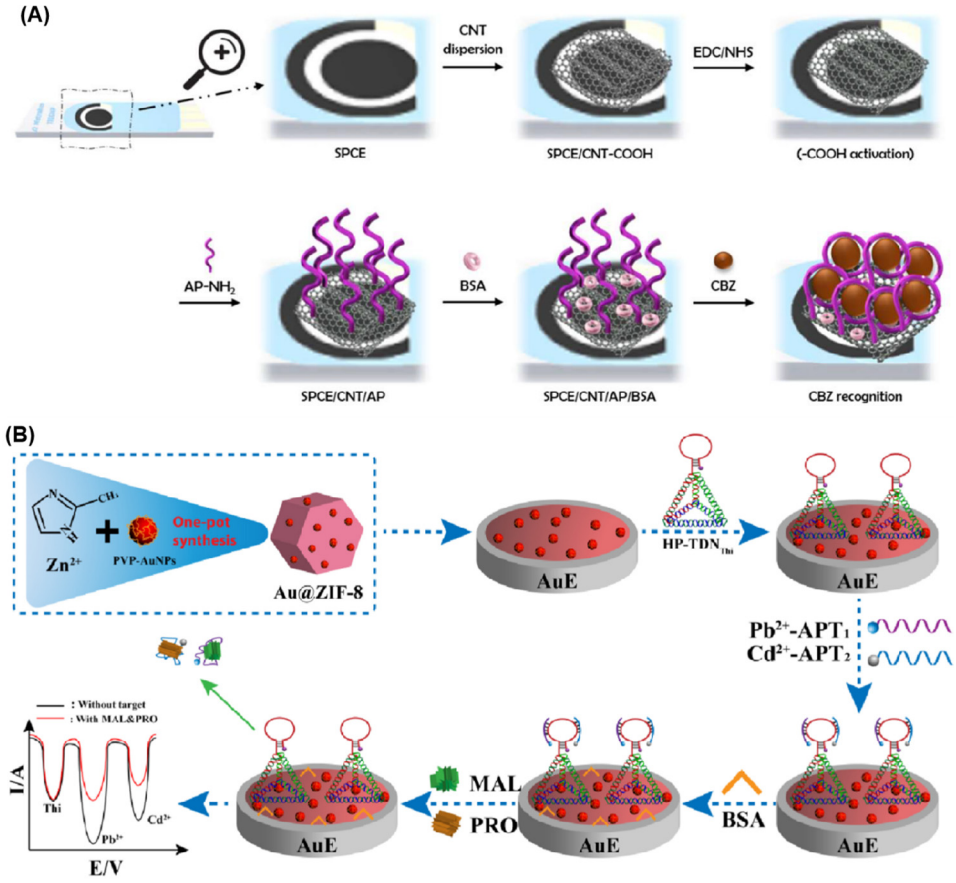


Figure 2. (**A**) The fabrication of a label-free electrochemical aptasensor for CBZ detection. (Reproduced with permission from Ref. [70] Copyright 2023, MDPI AG). (**B**) Scheme of the dual-ratiometric electrochemical aptasensing platform for the simultaneous detection of MAL and PRO. (Reproduced with permission from Ref. [71] Copyright 2023, Elsevier).

Electrochemical aptasensors also can simultaneously determine pesticides. Liu et al. designed a dual-ratiometric electrochemical aptasensor for the simultaneous detection of malathion (MAL) and profenofos (PRO) [71]. To obtain signal amplification, they firstly fabricated gold nanoparticle (AuNP)-encapsulated zeolitic imidazolate framework (ZIF-8) nanocomposites (Au@ZIF-8) to improve the immobilization amount of hairpin-tetrahedral DNA nanostructures (HP-TDN) and facilitate the electron transfer between the DNA probe and the electrode surface (Figure 2B). Then, metal ions (Pb^{2+} and Cd^{2+}) served as signal tracers to further amplify detection signal. Pb^{2+}-functionalized MAL aptamer (Pb^{2+}-APT1) and Cd^{2+}-functionalized PRO aptamer (Cd^{2+}-APT2) were co-assembled on the surface of thionine-labeled DNA nanostructures (HP-TDN_{Thi}) to specifically recognize MAL and PRO, respectively. With the addition of MAL and PRO, Pb^{2+}-APT_1 and Cd^{2+}-APT_2 dissociated from the surface of HP-TDN_{Thi}, leading to a decrease in both I_{Pb2+}/I_{Thi} and I_{Cd2+}/I_{Thi} oxidation current ratios. The detection limits of this aptasensor for MAL and PRO detection can reach 4.3 pg/mL and 13.3 pg/mL under optimized conditions, respectively. The obtained high performance suggested that this work may pave a way to develop aptasensors for multiple pesticides' detection in food safety and environmental monitoring.

3.3. Detection of Small Biological Molecules

Physiological metabolic activities of living organisms are closely related to various biological small molecules, such as neurotransmitters, adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NADH), hydrogen peroxide (H_2O_2), etc. [72–74]. Some small molecules including neurotransmitters and H_2O_2 can be directly detected by electrochemical sensors. However, direct detection is susceptible to interference from other electrochemical active molecules, resulting in low selectivity and sensitivity. Therefore, the biological recognition reaction is a promising tool to construct an electrochemical sensing platform for some biological small molecules' detection. According to this concept, electrochemical immunosensors and aptasensors have been widely developed to analyze ATP, dopamine (DA), uric acid (AA), 8-hydroxy-2′-deoxyguanosine (8-OHDG), etc. [75,76]. Compared with electrochemical immunosensors, electrochemical aptasensors attracted more and more attention due to their easy design, low cost, and controllable assembly.

Taking DA as an example, many electrochemical biosensors have been developed to directly determine it [77,78]. However, the co-existing AA, norepinephrine, adrenaline, and isoproterenol often affect the analytical performance of those designed biosensors. To improve the sensitivity and selectivity of electrochemical biosensors for dopamine detection, Zhang et al. used aptamer as a recognition unit, methylene blue-integrated m-PdNFs as a signal amplification unit, and Ce-MOF as an electrode-modified unit to construct a novel electrochemical aptasensor [79]. As shown in Figure 3A, a single-stranded DNA (S2) was assembled on the surface of the designed m-PdNFs-G4-MBs nanocomposites to obtain signal probes (m-PdNFs-S2-G4-MBs), which can offer electrochemical signals for detection. In the absence of DA, the anti-DA aptamer was hybridized with a single-stranded DNA (S1), which was further co-immobilized on the surface of the Ce-MOF nanocomposite-modified electrode. At this moment, the m-PdNFs-S2-G4-MBs signal probe cannot be assembled on the electrode surface via a DNA hybridization reaction. As a result, no electrochemical signal was obtained. In the presence of DA, DA preferentially binds with the aptamer to form the DA–aptamer complex, leading to the destruction of the aptamer-S1 double-strand structure. Therefore, the exposed S1 can be hybridized with the m-PdNFs-S2-G4-MBs signal probe to form double-stranded structures, generating significant electrochemical signals. Due to the extraordinary conductivity of Ce-MOF and the signal amplification of m-PdNFs-S2-G4-MBs, the designed electrochemical aptasensor achieved a low detection limit of 6 pM for DA detection. The practical DA detection result is highly consistent with the UPLC-MS method, suggesting that this aptasensor has high sensitivity and specificity.

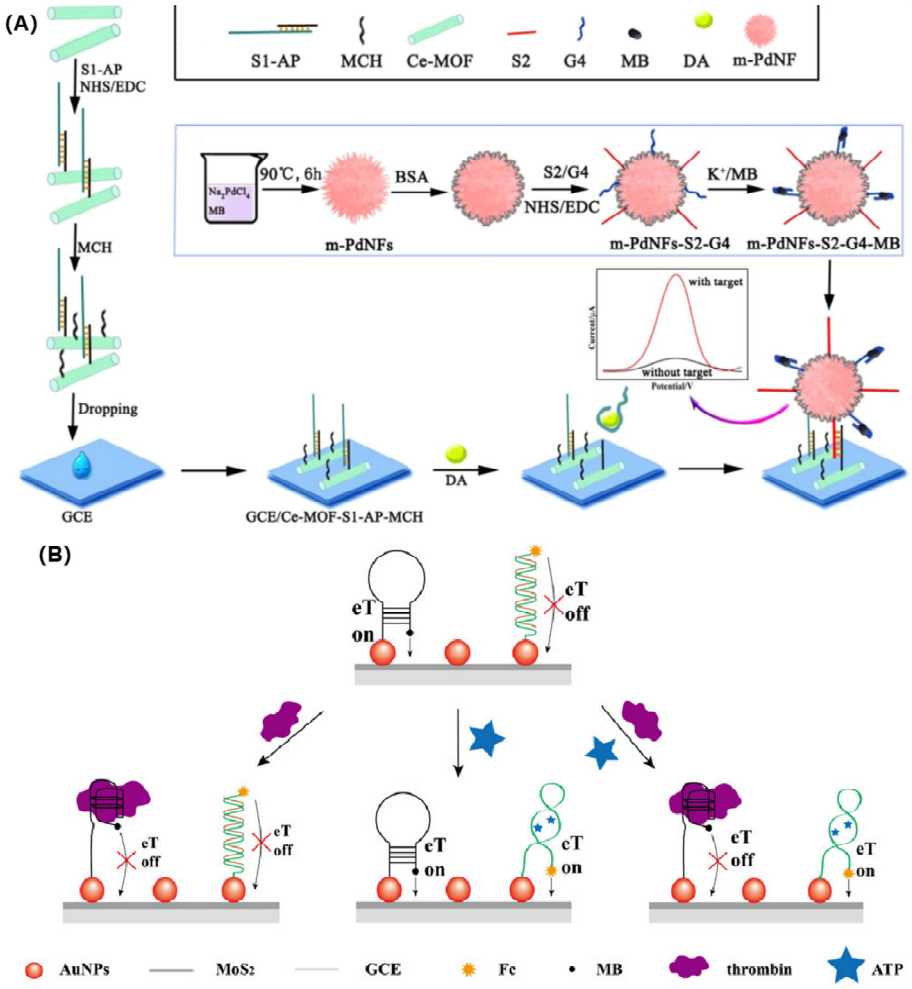


Figure 3. (**A**) Schematic diagram of a novel electrochemical aptasensor for dopamine detection based on MB-integrated m-PdNFs signal material. (Reproduced with permission from Ref. [79] Copyright 2022, Elsevier). (**B**) Schematic description of a dual-target electrochemical biosensor for ATP and thrombin detection based on DNA structural switching. (Reproduced with permission from Ref. [80] Copyright 2016, American Chemical Society).

Another example was given by Su et al. [80]. They constructed an electrochemical aptasensor for the simultaneous detection of ATP and thrombin (Figure 3B). They utilized the advantages of molybdenum disulfide (MoS_2) and noble metallic nanoparticles to synthesize MoS_2-based nanocomposites. After co-immobilizing anti-ATP and anti-thrombin aptamers on the surface of the MoS_2-based nanocomposite-modified electrode, a dual-target electrochemical aptasensor was constructed. Once the binding with ATP and thrombin is completed, the structures of two aptamers are changed, resulting in the labeled ferrocene (Fc) and methylene blue (MB) close to and far away from the electrode surface. Correspondingly, the electrochemical signal increased and decreased with the addition of ATP and thrombin, respectively. In other words, the designed aptasensor featured both "signal-on" and "signal-off" sensing mechanisms for the detection of ATP and thrombin, respectively. Under optimal conditions, this proposed aptasensor can simultaneously determine values as low as 0.74 nM ATP and 0.0012 nM thrombin. Furthermore, they employed the aptasensor to construct an "AND" logic gate platform for ATP and thrombin detection, which demonstrated a general methodology for the advancement of the highly sensitive and selective detection of various aptamer-specific binding targets.

3.4. Detection of Drug and Antibiotic Molecules

As we know, drugs and antibiotics greatly ensure the health of living organisms. However, the abuse of drugs and antibiotics, as well as their concentration-related toxicity, can endanger human health and even lives. Therefore, accurate, fast, and low-cost detection of drugs and antibiotics plays a vital role in disease diagnosis, treatment evaluation, and monitoring after operation.

An electrochemical aptasensor is also a powerful tool for the detection of drugs. For example, Chung et al. chose a de novo aptamer to develop an electrochemical aptasensor for the analysis of the anti-epileptic drug carbamazepine (CBZ) (Figure 4) [81]. The selected aptamer specifically binds with CBZ (K_d < 12 nM) to form a G-quadruplex structure, leading to the labeled MB close to the electrode surface. Correspondingly, the electron transfer between MB and the electrode is facilitated, increasing the electrochemical signal. It should be noted that the designed aptasensor has two functional modules: a 30 min assay for routine monitoring and a 5 min assay for rapid emergency testing. Under optimal conditions, this aptasensor exhibits a wide dynamic range (10 nM to 100 μM) and low detection limits of 1.25 nM and 1.82 nM for 5 min and 30 min analysis, respectively. The practical testing in the pricking blood sample (<50 μL) for CBZ determination proved the clinical applicability of this electrochemical aptasensor, suggesting that it has promising potential in point-of-care testing of personalized CBZ dosing. Using a similar sensing mechanism, cocaine, diclofenac, and amphetamine can be sensitively and selectively detected by electrochemical aptasensors without introducing other signal indicators [82–84].

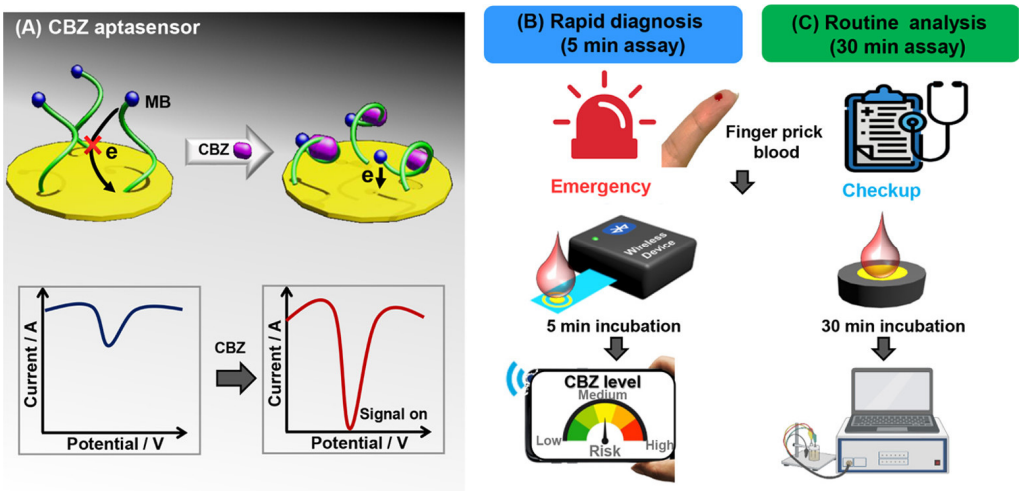


Figure 4. (**A**) Sensing schematic of the electrochemical aptasensor and its workflow for (**B**) 5 min rapid assay and (**C**) 30 min routine analysis for CBZ analysis. (Reproduced with permission from Ref. [81] Copyright 2022, American Chemical Society).

Besides drugs, electrochemical aptasensors are also widely employed to detect antibiotics [85]. A typical example is offered by Sun's group. They designed an electrochemical aptasensor for the ultrasensitive detection of neomycin by using a dual-signal amplification strategy [86]. In this sensing strategy, Fc-decorated multi-walled carbon nanotubes (MWCNTs) and the silica hybridized mesoporous ferroferric oxide nanoparticles (Fe_3O_4@SiO_2) were co-immobilized on the gold electrode surface to improve the electronic signal, which was used to load more RNA aptamers (Apt1) and facilitate electron transfer. Another RNA aptamer (Apt2) was assembled on the surface of gold nanoparticles to form a nanoprobe for signal amplification. In the presence of neomycin, a classical sandwiched structure was formed on the electrode surface, generating an obvious electrochemical signal. Due to the synergistic effect of different nanomaterials, this electrochemical aptasensor can detect 0.759 nM neomycin with high specificity and selectivity. Moreover, this aptasensor can efficiently determine neomycin in milk. Similarly, Emaminejad's, Wang's, Du's, and

Roushani's groups developed a series of electrochemical aptasensors for the detection of tobramycin [87], kanamycin [88], enrofloxacin [89], and streptomycin [90], respectively.

3.5. Detection of Proteins

Immunosensors coupled with different detection techniques have become universal tools for the detection of proteins, which have been widely applied in early diagnosis and clinical treatment of diseases [91]. However, antibody-based immunosensors have some disadvantages including the limited source of antibodies, high cost, disordered assembly on sensing interfaces, and the loss of biological activity [92], which greatly affect their detection performance and limit their further application. Therefore, electrochemical aptasensors have attracted more and more attention in protein detection due to their outstanding advantages, such as low cost, easy preparation, ordered assembly on the sensing interfaces, and anti-environment disturbance ability.

Highly efficient analytical approaches are of great significance for patients to realize early and rapid diagnosis, receive appropriate and timely treatment, and reduce mortality. Currently, C-reactive protein (CRP) has been identified as an angiocardiopathy special indicator for early diagnosis of cardiovascular disease with a high incidence rate [93]. Li and his colleagues have constructed an enzyme-free sandwich-type aptasensor for sensitive CRP detection based on $Mn_3(PO_4)_2$/CRP aptamer biomineralized nanosheets and polydopamine/Au nanospheres (PDA/Au NPs) (Figure 5A) [94]. The designed $Mn_3(PO_4)_2$/CRP aptamer biomineralized nanosheets are not only used as recognition units for capturing target CRP, but also serve as signal enhancers to improve electrochemical responses. Coupled with the signal amplification nanoprobe of PDA/Au NPs, the aptasensor exhibits excellent detection performance with a wide linear range (1 pg/mL–1 ng/mL) and a low detection limit (0.37 pg/mL). The repeatability, specificity, and reliability of this aptasensor are superior to that of the traditional immunoturbidimetry assay, suggesting that it has great prospects in clinical diagnostic applications of cardiovascular disease. Vascular endothelial growth factor (VEGF) is a key biomarker of diabetic retinopathy (DR), which can be also determined by electrochemical aptasensors [95]. For instance, Mei et al. designed a noninvasive electrochemical aptasensor to detect VEGF in tears [96]. They developed a cascade signal amplification strategy by combining a hybridization chain reaction (HCR) with CeO_2 nanoparticles to improve the analytical performance (Figure 5B). The introduced CeO_2 nanoparticles can efficiently catalyze H_2O_2 to generate electrochemical signals. Under optimal conditions, the aptasensor can detect 1 fg/mL~0.1 ng/mL VEGF with a detection limit of 0.27 fg/mL. Based on the acceptable stability and reproducibility, the proposed aptasensor can accurately analyze VEGF in tears comparable to the ELISA method, indicating that it is a potential tool for early diagnosis and monitoring of DR. Similarly, Li's, Hosseini's, and Gao's teams have constructed various electrochemical sensing platforms coupled with aptamers and a signal amplification strategy for different protein biomarkers' detection, including thrombin [97], tumor necrosis factor α [98], and tau protein [99], respectively.

Electrochemical aptasensors have also been widely used to monitor infectious diseases, such as SARS-CoV-2 [100], malaria [101], tuberculosis [102], etc. As shown in Figure 5C, Idili et al. have developed a simple electrochemical aptasensor for the detection of SARS CoV-2 S protein in undiluted samples (serum and artificial saliva) [103]. Typically, the added SARS CoV-2 S protein induced the change in the methylene blue derivative-labeled aptamer conformation, resulting in the electrochemical indicator close to the electrode surface. Correspondingly, a concentration-related electrochemical signal is generated for the qualitative and quantitative detection of SARS CoV-2 S protein. This electrochemical aptasensor is simple (single step) and fast (detection time < 5 min), suggesting that it is an ideal device for COVID-19 monitoring. Another typical example was offered by Rahmati et al. They constructed a label-free electrochemical aptasensor for the analysis of SARS-CoV-2 spike glycoprotein based on a copper hydroxide nanorod-modified screen-printed carbon electrode (SPCE) [104]. The obtained SARS-CoV-2 spike glycoprotein–

aptamer complex blocked the electron transfer, leading to a decrease in the electrochemical signal. Based on this phenomenon, the designed aptasensor can efficiently detect SARS-CoV-2 spike glycoprotein in buffer, labeled saliva samples, and actual clinical samples. It is noted that the analytical performance of this aptasensor is comparable to the PCR results, proving that it has great potential in rapid and on-site COVID-19 monitoring.

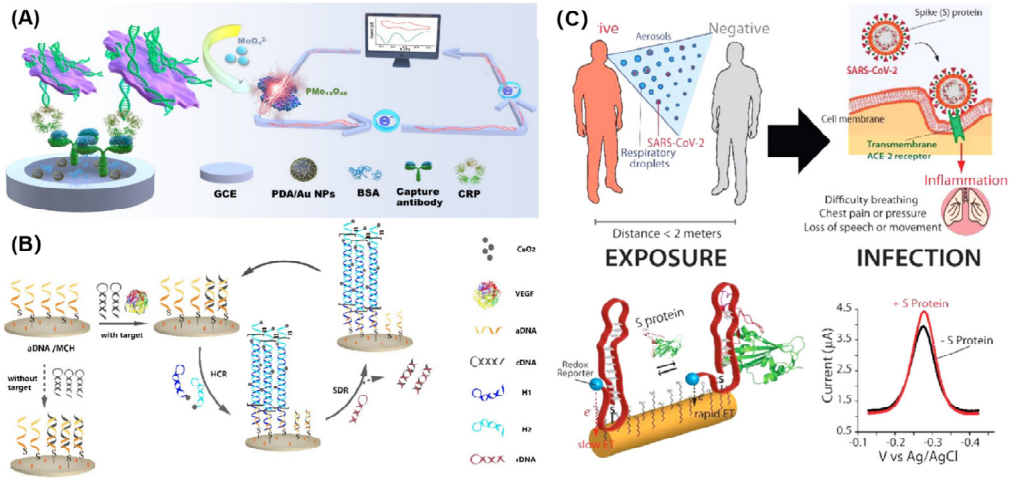


Figure 5. (**A**) The preparation of the $Mn_3(PO_4)_2$/aptamer hybrid nanosheet−based electrochemical aptasensor for CRP detection. (Reproduced with permission from Ref. [94] Copyright 2021, Elsevier). (**B**) Schematic diagram of electrochemical aptasensor coupled with signal amplification strategy for sensitive detection of VEGF. (Reproduced with permission from Ref. [96] Copyright 2021, Elsevier). (**C**) Schematic of electrochemical aptasensor for the rapid and efficient detection of the SARS-COV-2 spike protein. (Reproduced with permission from Ref. [103] Copyright 2021, American Chemical Society).

3.6. Detection of Exosomes

As nanoscale extracellular vesicles, exosomes play an important role in promoting tumor growth, migration, invasion, and information transfer between cells [105]. Therefore, exosomes are considered as excellent biomarkers for early diagnosis of cancer [106]. However, it is still a challenge to sensitively and selectively detect exosomes due to their low content in biological fluids during the early stages of cancer [107,108]. In recent years, electrochemical aptasensors have been gradually employed to detect exosomes due to their advantages. Coupled with signal amplification strategies, the analytical performance of electrochemical aptasensors can meet the need for exosome detection [109].

Zhang's group developed an electrochemical aptasensor for exosome detection based on a microelectrode and HCR amplification strategy [110]. They introduced EpCAM aptamers to selectively recognize and capture EpCAM-positive cancerous exosomes. Due to the HCR reaction, a large number of avidin–horseradish peroxidase (HRP) molecules were assembled on the microelectrode surface, which can catalyze the 3,3′,5,5′-tetramethylbenzidine (TMB)+H_2O_2 reaction strategy to generate a large electrochemical signal. Due to the synergetic effect of the enzyme catalytic reaction, HCR reaction, and microelectrode, this aptasensor has a detection limit as low as 5×10^2 exosomes per milliliter. Moreover, this aptasensor can successfully detect cancerous extracellular vesicles in serum samples of early- and late-stage lung cancer patients. Chang et al. used programmed cell death ligand 1 protein-positive (PD-L1+) exosome as a biomarker to evaluate the diagnosis of non-small cell lung cancer (NSCLC) [111]. They integrated the specific recognition ability of the aptamer, the excellent peroxidase-like catalytic activity of palladium–copper–boron alloy microporous nanospheres (PdCuB MNs), and the high conductivity of Au@$CuCl_2$ nanowires (NWs) to construct an electrochemical aptasensor for detecting PD-L1+ exosomes (Figure 6A). In the presence of PD-$L1^+$ exosomes, a classical sandwiched structure was formed on the electrode surface. The introduced PdCuB

MNs efficiently catalyze H_2O_2 to generate a significant electrochemical signal. According to this result, the aptasensor can detect 1×10^2–1×10^8 particles/mL exosomes with a low detection limit of 36 particles/mL. Results obtained from clinical samples suggested that this aptasensor has a potential application in the early diagnosis of NSCLC.

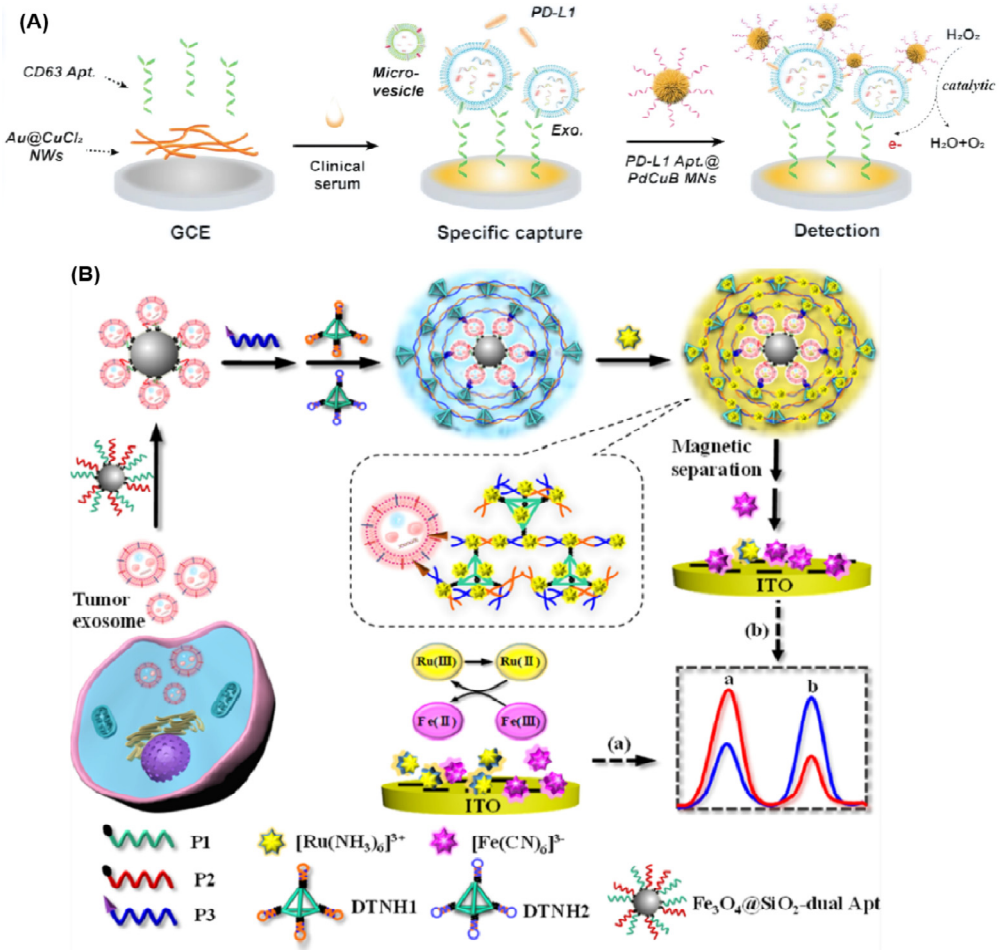


Figure 6. (**A**) Schematic illustration of a nanomaterial-based electrochemical aptasensor for PD-L1$^+$ exosome analysis. (Reproduced with permission from Ref. [111] Copyright 2023, Springer Nature). (**B**) Schematic illustration of a ratiometric immobilization-free electrochemical aptasensor for precise capture and direct quantification of (a) in the absence and (b) in the presence of the tumor exosomes. (Reproduced with permission from Ref. [112] Copyright 2021, American Chemical Society).

Besides captured probes' immobilized mode, Li et al. developed a dual-aptamer recognition system for precise capture and direct analysis of tumor-specific exosomes without immobilization [112]. They assembled both CD63 and mucin 1 (MUC1) aptamers onto the surface of amino-functionalized Fe_3O_4@SiO_2 nanoparticles to construct dual-aptamer-modified nanoprobes (Fe_3O_4@SiO_2-dual Apt). As shown in Figure 6B, the dual-aptamer-modified nanoprobes can specifically capture target exosomes due to the recognition ability of two aptamers. After magnetic separation, cholesterol-labeled DNA probes (P3) can anchor on the surface of exosomes and activate the hyperbranched hybridization chain reaction, generating a magnetic bead–exosome–hyperbranched DNA superstructure (Fe_3O_4@SiO_2-exosome-HDS). This superstructure can adsorb a large number of $Ru(NH_3)_6^{3+}$(Ru (III)) molecules, resulting in a few unbound Ru (III) remaining in the supernatant after magnetic separation. Therefore, the redox reaction between Ru (II) and $[Fe(CN)_6]^{3-}$ (Fe (III)) is inhibited, leading to a significant increase in $I_{Fe(III)}/I_{Ru(III)}$. The ratio of the electrochemical signal was related to exosomes, which can be used to quantitatively and qualitatively analyze target exosomes. Consequently, this ratiometric dual-signal elec-

trochemical aptasensor exhibits high reliability, accuracy, and easy manipulation, which can sensitively detect tumor exosomes in complex environments and human serum samples.

3.7. Detection of Tumor Cells

It is well known that the incidence rate and mortality of cancer have increased year by year, which has brought a huge threat to human health. Thus, the identification and detection of cancer cells in patients' tumor lesions and circulating blood is crucial for early clinical diagnosis, toxicity monitoring, and public health protection [113,114]. However, conventional clinical, imaging, and other diagnostic methods often have weak sensitivity and specificity due to the complex etiology of tumors, leading to misdiagnosis and missed diagnosis [115,116]. Although a variety of technologies including PCR-based methods [117], cell counting methods [118], and fluorescence determination [119] have been presented, many disadvantages of these methods have limited their wide application, such as expensive fluorescent labeling reagents, cumbersome processes, and the need for advanced instruments and professionals. Therefore, it is necessary to develop reliable, cost-effective, sensitive, and easy to be clinically popularized detection tools for high-precision diagnosis of cancer cells.

Electrochemical aptasensors have received widespread attention in the high-performance detection of tumor cells due to their significant advantages, such as high sensitivity, simplicity, rapid response, reusability, and low cost [120]. An impressive example was given by Cai et al. [121]. As shown in Figure 7A, a double-stranded DNA formed by a biotin-labeled aptamer and DNA walker was assembled on the surface of the streptavidin-modified magnetic microsphere to construct nanoprobe, which was used to recognize and capture target cancer cells. Once capturing target breast cancer MCF-7 cells, an MCF-7–aptamer complex was formed and a DNA walker was released from the surface of the nanoprobe. The released DNA walker (DNAzyme) repeatedly cleaved D-RNA with the help of Mg^{2+} to produce a large number of short nucleic acids, leading to a great decrease in electrochemical signals. With DNAzyme-assisted signal amplification, the designed aptasensor can analyze as low as 47 cells/mL MCF-7 cells. G-quadruplex DNAzyme is also widely used for signal amplification to construct electrochemical aptasensors for cancer cell detection. Chen and co-workers designed a super sandwich G-quadruplex DNAzyme to construct an electrochemical aptasensor for the highly sensitive detection of cancer cells [122]. In Figure 7B, amino-modified aptamers (S1) and their complementary DNA sequence (S2) were immobilized onto the surface of magnetic beads (Fe_3O_4 NPs) to form S2/S1/Fe_3O_4 conjugates, which were used to specifically capture K562 cells. Once encountering K562 cells, the double-stranded S1–S2 structure was dissociated due to the stronger affinity between K562 cells and S1. As a result, S2 was released from the surface of Fe_3O_4 NPs and hybridized with a capture probe (S3) on the electrode surface. The S2–S3 structure triggered a hybridization cascade between S4 and S5, resulting in the super sandwich structure. In the presence of Hemin, a large number of G-quadruplex DNAzymes were formed on the electrode surface. Finally, the G-quadruplex DNAzyme catalyzes the reduction of H_2O_2 by MB, leading to a dramatically amplified electrochemical signal. Under optimal conditions, the detection limit of the as-fabricated aptasensor was estimated to be 14 cells/mL K562 cells.

Electrochemical aptasensors are also extensively employed to detect circulating tumor cells (CTCs), which are of great significance for the clinical diagnosis, metastasis, and prognosis of tumors [123,124]. Shaegh et al. reported a novel microfluidic electrochemical aptasensor by coupling a microchip with a screen-printed gold electrode for detecting A549 cells, which is considered as a CTC model [125]. As shown in Figure 7C, the added A549 cells were specifically bound with the aptamer to form a complex on the electrode surface, which blocked the electron transfer between $[Fe(CN)_6]^{3-/4-}$ and the electrode surface. As a result, the electrochemical signal is decreased with the increasing concentration of A549 cells. According to this phenomenon, this biosensor can sensitively detect 50~5 $\times$ 10^5 cells/mL A549 cells with a low detection limit of 14 cells/mL. More importantly, this microfluidic aptasensor can effectively determine A549 cells in complex

matrices (such as human serum), providing that this sensing platform can be used to detect low-abundance biomarkers obtained from liquid biopsies.

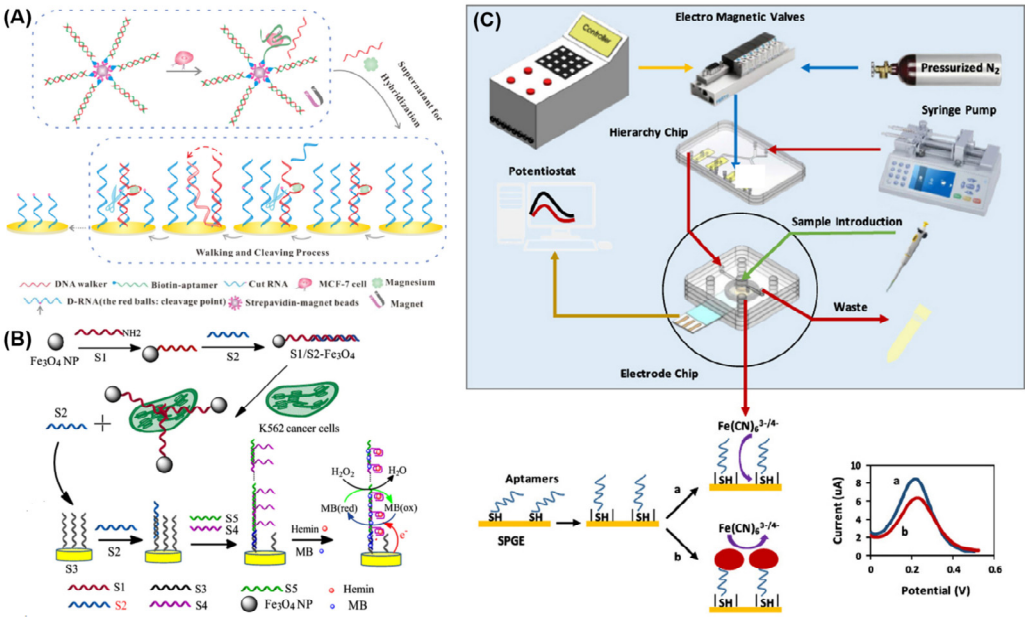


Figure 7. (**A**) Schematic illustration of the electrochemical aptasensor for MCF-7 cells' detection based on a DNA walker signal amplification strategy. (Reproduced with permission from Ref. [121] Copyright 2016, Elsevier). (**B**) Schematic representation of signal-amplified G-quadruplex DNAzyme for ultrasensitive detection of K562 cells. (Reproduced with permission from Ref. [122] Copyright 2015, Elsevier). (**C**) Schematic illustration of microfluidic electrochemical aptasensor and its electrochemical signal (a) in the absence of and (b) in the presence of A549 cells. (Reproduced with permission from Ref. [125] Copyright 2023, Elsevier).

3.8. Detection of Microbial Pathogens

Since the COVID-19 pandemic, infectious diseases have gained people's attention. Accurate and fast detection of pathogenic microorganisms is of great significance in controlling and preventing microbial infectious diseases. At present, direct smear microscopy, isolation and culture, serological reaction, and PCR are the main methods for the rapid identification and detection of pathogenic microorganisms [126]. However, some limitations of these methods exist, such as being time-consuming, requiring professional personnel and instruments, having low sensitivity, and so on. Therefore, it is urgent to develop simple, rapid, sensitive, and on-site methods for the detection of microbial pathogens.

To meet these requirements, a series of electrochemical aptasensors have been developed for the high-performance analysis of microbial pathogens due to their outstanding advantages. Bian et al. have constructed an electrochemical aptasensor for highly selective and ultrasensitive detection of single-cell levels of bacteria [127]. In Figure 8A, Au NPs modified with aptamers and 6-(Ferrocenyl) hexanethiol molecules (Au@Fc-Apt) and bacteria-imprinted polymer films (BIFs) were employed as the signal nanoprobe and the capture probe, respectively. In the presence of *Staphylococcus aureus* (*S. aureus*), a classical sandwiched structure was formed on the electrode surface. As a result, an obvious electrochemical signal is generated from the assembled Au@Fc-Apt signal nanoprobe, which can be used to qualitatively and quantitatively detect bacteria. In view of this, the aptasensor could detect single-cell level and 10 CFU mL^{-1} *S. aureus* in an ideal buffer and a complex milk sample, respectively. Moreover, this proposed aptasensor can efficiently distinguish *S. aureus* from the multiple coexisting interfering bacteria, suggesting that this aptasensor has outstanding selectivity.

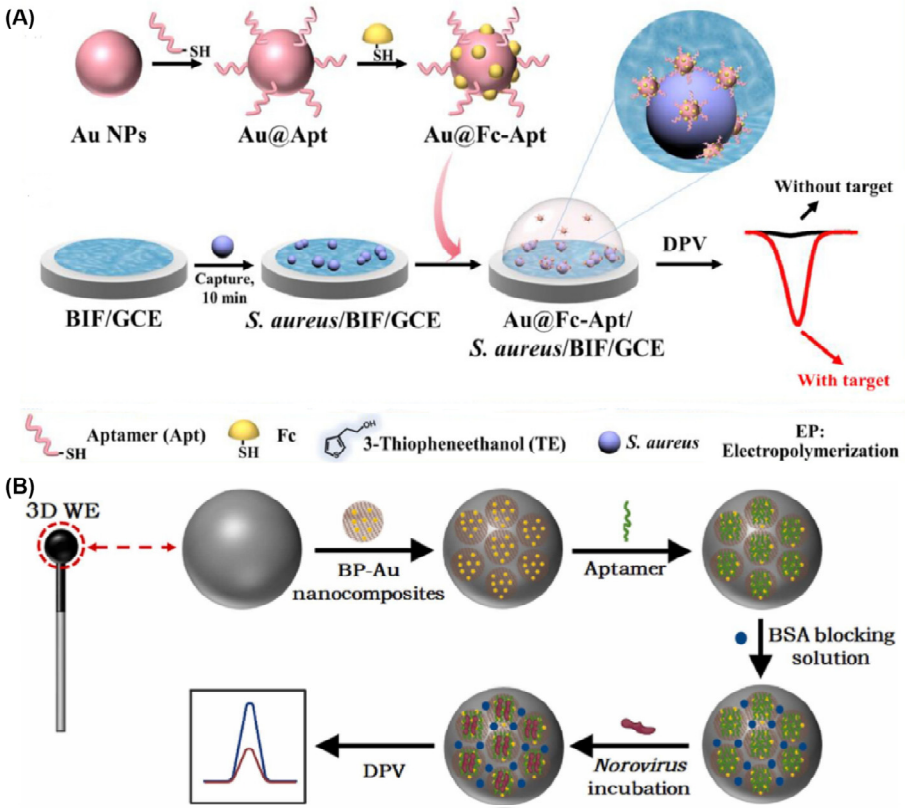


Figure 8. (**A**) Schematic preparation of the electrochemical aptasensor for highly selective and ultrasensitive detection of *S. aureus*. (Reproduced with permission from Ref. [127] Copyright 2023, American Chemical Society). (**B**) Workflow scheme for the electrode functionalization and aptasensing of norovirus based on BP-AuNCs. (Reproduced with permission from Ref. [128] Copyright 2022, Elsevier).

Besides bacteria detection, electrochemical aptasensors have also been widely used to detect viruses. For fast identification and detection of norovirus, Jiang et al. developed a 3D electrochemical aptasensor for sensitive detection of norovirus based on a movable spherical working electrode (WE) decorated with phosphorene–gold nanocomposites (BP-AuNCs) [128] (Figure 8B). The design of a movable spherical WE is beneficial for increasing surface area, simplifying sampling, and avoiding cross-contamination. The added norovirus specifically binds with aptamer and induces the change in aptamer structure, leading to the decrease in the electrochemical signal. As expected, the 3D electrochemical aptasensor can detect 1 ng/mL–10 μg/mL norovirus with a detection limit of 0.28 ng/mL. The recovery of this aptasensor for norovirus detection in spiked oyster samples ranges from 97.2% to 103.7%, suggesting that this aptasensor has a potential practical application. The proposed aptasensor for norovirus detection provides a facile, low-cost, highly sensitive, and selective platform, which is potentially applied in the fields of food safety and clinical diagnosis. Besides norovirus, avian influenza virus [129], SARS-CoV-2 virus [130], and dengue virus [131] have also been analyzed by electrochemical aptasensors.

4. Prospects and Challenges of Electrochemical Aptasensors

Nowadays, electrochemical aptasensors have achieved great advances in environmental monitoring, drug safety, biochemical analysis, disease diagnosis, and public safety due to their outstanding advantages of fast response, simple preparation, easy design, high specificity for chemical and biological molecules, excellent chemical stability, and low cost. Inspired by the above exciting progresses, integrating electrochemical aptasensors into portable, wearable, and implantable devices has become a popular trend for their application in POCT and real-time monitoring fields, which has the potential to completely

change diagnosis and health management, especially in application scenarios for home users or remote areas where it is difficult to receive hospital nursing care [132]. In addition, with the fast development of nanotechnologies and miniaturization technologies, as well as the urgent need for various detection strategies, electrochemical aptasensors have broad applications and bright prospects in the future.

For achieving these goals, some challenges of electrochemical aptasensors should be solved: (1) For broad applications, more aptamers should be selected, which play a vital role in the detection of various chemical and biological molecules. Nowadays, hundreds of aptamers have been selected to specifically recognize target chemical/biological molecules. However, it is not enough for sensing/biosensing applications in many fields. Therefore, the selection of more aptamers with high binding affinity can promote the development of electrochemical aptasensors. (2) The integration of cutting-edge innovative technologies (e.g., DNAzyme, CRISPR, microfluidic chips, etc.) into electrochemical aptasensors is very prospective to design novel aptasensor devices, which can efficiently enlarge their application [133]. (3) The construction of suitable signal amplification strategies is a vital effect factor to construct high-performance electrochemical aptasensors. Up to now, several signal amplification strategies have been used to construct electrochemical aptasensors, such as loop-mediated isothermal amplification, rolling circle amplification (RCA), HCR, catalyzed hairpin assembly (CHA), enzyme-assisted signal amplification, and nanoprobe-based signal amplification, which can efficiently amplify the detection signal and reach lower detection limits [134]. Therefore, how to choose a signal amplification strategy greatly depends on the analytes and application scenarios. (4) For the POCT and real-time monitoring applications, it is still a challenge to construct a portable and intelligent electrochemical aptasensor by combining the detection instruments and signal communication technologies. To quickly keep up with global health issues, electrochemical aptasensors should gradually being clinically transformed and adapted to market demands. Therefore, the development of home-care, low-cost, real-time, and portable devices has become a popular and attractive research field [135,136]. In a word, electrochemical aptasensors will be an ideal sensing platform for sensitive, selective, real-time, and on-site detection of target molecules in complex environments.

Author Contributions: Conceptualization, R.Y. and S.S.; methodology, R.Y. and J.C.; formal analysis, J.C. and H.M.; investigation, R.Y., H.M., and Y.L.; writing—original draft preparation, R.Y.; writing—review and editing, S.S. and Y.L.; supervision, S.S. and L.W.; project administration, S.S. and L.W.; funding acquisition, L.W. and S.S. All authors have read and agreed to the published version of the manuscript.

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