Chapter 1

Recent Advances in the Use of Nucleic Acid Aptamers in Electrochemical and Optical Biosensors

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Abstract

Nucleic acid aptamers are single-stranded DNA (ssDNA) or RNA oligonucleotides that have the ability to recognize different targets with their special secondary structures. Aptamers, which have high specificity and affinity, are gaining increasing importance in biosensor technologies as recognition elements that can be easily synthesized chemically and modified according to the need. The small size, stability, easy modification and low immunogenicity of aptamers make them an important alternative to traditional antibodies as biosensor recognition elements. In this book chapter, current electrochemical and optical approaches to aptamer-based biosensors are comprehensively discussed and systems based on various sensing principles are comparatively analyzed. Particular attention is paid to electrochemical, colorimetric, fluorescent, surface plasmon resonance (SPR) and lateral flow assay (LFA)-based aptasensor designs. This chapter compares different biosensor platforms in terms of sensitivity, selectivity and analytical parameters, and also includes technological advances that enable the development of new generation aptasensors. In this context, the study aims to be an up-to-date reference source for future research in the field of aptasensors.

1. Introduction

Nucleic acid aptamers are unique recognition elements that have shown great potential for use in numerous applications since they were first described by Ellington and Szostak (1). Nucleic acid aptamers, consisting

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of short-chain RNA or ssDNA sequences, are commonly developed against almost any target by the method called Systematic Evolution of Ligands by Exponential Enrichment (SELEX) (2). During the development of aptamers using the SELEX method, environmental conditions such as ionic strength, temperature, and pH can be adjusted as needed. This makes aptamers very advantageous as recognition elements, as they can be manipulated according to the desired conditions. The main advantages of aptamers over antibodies, which are traditionally the most preferred recognition elements in biosensors, are their chemical synthesizability, high specificity and affinity, small size, stability and durability, and easy modifiability. Moreover, the batch-to-batch variability of aptamers is lower than that of antibodies (3). In particular, their ability to be easily modified while preserving their secondary structures has brought aptamers to the forefront as recognition elements in biosensors over the past decade (4). Biosensors developed using aptamers are becoming increasingly important in modern diagnostic technologies due to their high specificity and sensitivity. Various sensing principles such as optical, electrochemical, colorimetric, fluorescence-based, surface plasmon resonance (SPR), and piezoelectric can be easily adapted to aptasensors (5, 6, 7, 8, 9). Aptasensors are widely used in the determination of many different analytes such as clinical biomarkers, food toxins, environmental pollutants, pharmaceuticals, and small molecules due to their relatively low cost, high sensitivity, and fast response time. The development of aptasensors using different measurement strategies is closely related to the advantages of aptamers such as stability, high selectivity, easy modifiability, and reusability.

This chapter presents a comparative analysis of current approaches to nucleic acid aptamer-based biosensors. The focus is on the application of aptamers in various fields such as clinical biomarker diagnosis, food analysis, and environmental detection. Technological developments used in the design of aptasensors based on different sensing principles are also included. Different platforms are compared in terms of sensitivity, selectivity, and ease of application, accompanied by current examples from the literature.

2. Aptasensors

2.1. Electrochemical aptasensors

Electrochemical-based biosensors are analytical devices based on the principle of converting biochemical interactions between biological recognition elements and the surface of electrodes or electroactive components into electrical signals via a transducer (10, 11). They generally

operate with high sensitivity to specific target analytes using biologically based recognition elements such as enzymes, antibodies, nucleotides, molecularly imprinted polymers (MIPs), aptamers or cells. Electrochemical-based approaches, which can have different measurement principles such as amperometric, potentiometric, voltammetric and impedimetric, are widely used for the detection of analytes in a wide range of scales by offering advantages such as high sensitivity, low detection limit and short analysis time. Thanks to advanced surface modification techniques, it has become possible to immobilize biomolecules on the electrode surface in a way that they maintain their stable and interaction-friendly structures. With these features, electrochemical biosensors have gained an important place in commercial applications as well as maintaining their popularity in academic research. In recent years, aptamers have begun to play an important role as recognition elements in electrochemical biosensor designs. Particularly, the fact that aptamers can be modified at different positions with many different functional groups such as amine, thiol, carboxylic acid, biotin (12) during the chemical synthesis stage offers significant advantages in terms of surface design. The ability to modify aptamers with different functional groups is quite useful in terms of controlled surface immobilization. However, different strategies are required to orient antibodies (13). Aptamers can be covalently immobilized and directed to surfaces with different functionalities using modifications. In particular, it is possible to add linker sequences in addition to these modifications to provide ideal rational freedom for secondary structures related to target binding. A schematic diagram depicting the most commonly used electrochemical aptasensor techniques is presented in Figure 1. This diagram represents aptamer-modified electrodes that interact with target molecules and the electrochemical methods used to measure this interaction, such as EIS, SWV, and DPV.

In a recent study conducted by Didarian et al., an impedimetric biosensor was developed using the PTH (53–84) aptamer, in particular, as a result of the selection of different segment-specific aptamers for parathyroid hormone (PTH) by the SELEX method. In the study where the interface impedance change formed on the Carbon Screen Printed Electrode (SPE) electrode surface as a result of the interaction of the developed PTH fragment-specific aptamer with the target molecule was monitored, pg/mL sensitivity was achieved. This study also provides insight into the usability of aptamer-based biosensors in the instantaneous evaluation of surgical processes, focusing on a clinically important area such as intraoperative PTH monitoring (14). In another study based on impedimetric measurement, cerium oxide nanoparticles functionalized graphene oxide (CeO_2-GO) was modified with

target-specific aptamer for the detection of breast cancer biomarker EGFR. The developed aptasensor showed an extremely low detection limit of 1.87 fg/mL in PBS medium. The large surface area and high electrochemical conductivity of CeO_2-GO nanocomposite facilitated aptamer binding, thus increasing the sensitivity of the sensor (15). The study by Moradi and his colleagues has brought the first electrochemical impedimetric aptasensor developed for the detection of tramadol (TRA) to the literature. It is one of the most recent and striking examples in the field in terms of visual and quantitative verification of aptamer-target interaction with molecular dynamics (MD) simulation. The determination of the aptamer binding site by MD simulations provides an innovative approach in terms of design (16). A dual-mode aptasensor based on electrochemical impedance spectroscopy (EIS) and electrochemical (EC) was developed for the determination of aflatoxin B1 (AFB1), an important food toxin. The sensor was constructed by loading platinum and gold nanoparticles (MXene\@Thi/PtAuNPs) onto thionine-functionalized Ti_3C_2Tx MXene, and both electron transfer efficiency and internal reference signal providing capacity were increased. This nanocomposite structure was modified with hairpin-shaped DNA (hDNA) and ferrocene-labeled AFB1 aptamer (Fc-Apt) to transform it into a platform that provides specific signal changes in the presence of target. The sensor detected AFB1 with high accuracy and selectivity between 10 pg/mL and 30 ng/mL in ratiometric EC mode and between 3 pg/mL and 30 ng/mL in EIS mode. The study also yielded successful results in real corn samples, and the selectivity, repeatability and stability of the system were found to be high (17). The AFB1 aptamer was used as an electroactive signal transmitter by labeling it with ferrocene. When the AFB1 target is bound, the ferrocene-labeled aptamer leaves the general structure, thus the ferrocene signal (IFc) at the electrode decreases while the thionine signal increases, and this creates a sensitive detection mechanism. Although redox active molecules such as ferrocene do not directly produce a signal in the EIS analysis, they affect the charge transfer resistance (Ret) through the steric and electrical changes they create on the electrode surface, leading to a change in the EIS signal. Since the binding or dissociation of the ferrocene-labeled aptamer changes the charge transfer properties on the surface, it also provides an indirect but significant effect on the presence of the target in the EIS measurement. In this way, ferrocene-labeled aptamers contribute to target-specific and reliable signal changes in both voltammetric and impedimetric systems. In a recent study, which is an important example of the successful detection of large targets such as pathogenic cells with electrochemical aptasensors, *Escherichia coli* (*E. coli*) was detected in buffer with a detection limit of 1.4

CFU/mL. The impedimetric based aptasensor developed for the detection of E. coli bacteria in real samples was formed by covalently binding the DNA aptamer called P12-55 to a gold electrode and gave a linear response in the range of 100–10^4 CFU/mL. The sensor also showed successful results in real samples such as urine and tap water, maintaining high specificity and sensitivity especially in urine. The absence of cross-reactivity against other bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* proved its bioselectivity (18). In a study conducted by Sharma et al., a specific and sensitive aptamer-based impedimetric biosensor was developed for the diagnosis of chikungunya virus (CHIKV). The electrode surface was coated with nanographene oxide (NG) composite modified with zinc oxide (ZnO) nanoparticles to improve electron transfer and provide more aptamer retention. When the aptamers immobilized on this modified surface bound CHIKV antigen, electron transfer was inhibited and this was successfully detected by electroimpedimetric measurements. The developed aptasensor can detect antigen at a low limit of 1 ng/mL and gives a linear response up to 10 μg/mL; its performance and stability were also demonstrated in human serum. The absence of cross-reactivity against DENV antigen confirms the high specificity of the aptamer and the clinical selectivity of the sensor. The electrochemical aptasensor developed in this study is built on a paper-based platform, the electrochemical paper-based analyzer (ePAD). Such platforms are highly suitable for diagnostic applications, especially in resource-limited areas, due to their low-cost production, biodegradability, portability, and simple use (19). In one of the interesting studies conducted recently, a high specificity aptamer for rapid and portable dose determination of acute radiation sickness was developed by the MCP-SELEX method. Aptamers with high affinity for p21 protein were integrated into SPEs to form an EIS-based aptasensor. The developed sensor provides a linear response to radiation dose in the range of 0–10 Gy, while providing much faster and portable measurements than traditional methods with a low detection limit of 0.38 Gy. The study showed that the p21 protein level increased directly proportionally to the radiation dose, proving that this sensor works effectively both in cell cultures and in human peripheral blood samples. As a result, this method offers a new radiation dose assessment approach that is faster, portable and applicable in the field compared to existing biodetection techniques (20). Measuring the level of a radiation-sensitive protein (p21) with an aptamer-based electrochemical biosensor as in this study is a very rare strategy.

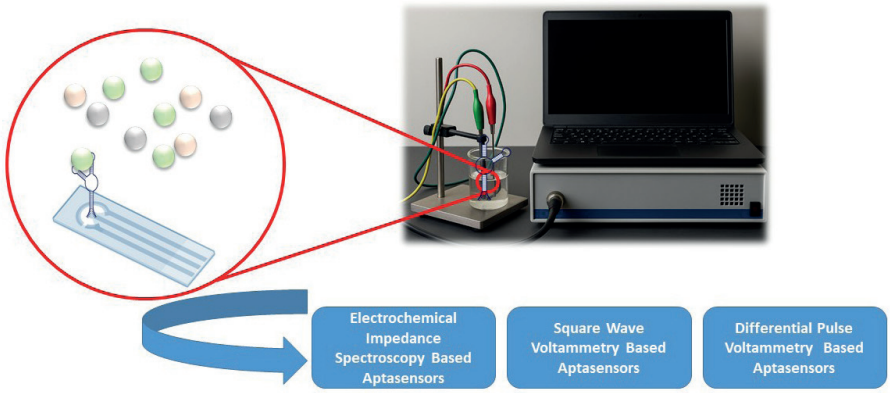

Figure 1. Schematic representation of electrochemical aptasensors. Target molecules bind to aptamer-modified electrodes, and the resulting signal is measured using electrochemical techniques such as electrochemical impedance spectroscopy (EIS), square wave voltammetry (SWV), and differential pulse voltammetry (DPV).

Square wave voltammetry (SWV) is an advanced electroanalytical technique characterized by high sensitivity and low background current, allowing rapid and selective detection of electroactive species depending on analyte concentration. A specific square wave voltammetry (SWV) based aptasensor for the hormone 11-deoxycortisol (11-DCL) was developed by comparing the MAB phase (MoAlB) and the multilayer MBene structures derived from it. MBene, obtained by removing aluminum from the MAB phase, provided a more suitable platform for aptamer immobilization by providing higher surface area and electron transfer capacity. The MBene-based sensor reached an extremely low detection limit of 0.014 pg/mL and showed high specificity against 11-DCL. The sensor gave minimal signals in cross-reactivity tests with similar hormones and achieved successful results in real serum samples. These findings indicate that MBene structures have high potential in electrochemical biosensors (21). MBene material, which has rarely been studied in the literature, was used for the first time in an electrochemical aptasensor platform in this study. While MXenes have been studied more traditionally, the use of MBenes in biosensor applications is limited. In this respect, the study pioneers the introduction of a new material to biosensor technologies. The effect of the MBene structure is evaluated in comparison with its direct precursor, the MoAlB MAB phase, and the advantages of MBene are demonstrated by electrochemical data. This approach provides a strong scientific contribution to the study from both mechanistic and engineering perspectives. In another study using the SWV method, DNA aptamers selected by the SELEX method were

used as recognition elements for the sensitive determination of the surface antigen SAG1 of Toxoplasma gondii. The sensor platform was created by immobilizing the SOK14 aptamer with high affinity specific to SAG1 onto screen-printed carbon electrodes modified with graphene quantum dots (GQD). Linear responses between 0.01–100 nM were obtained by square wave voltammetry and a low detection limit of 11.5 pM was achieved. Selectivity studies showed that there was no cross-reactivity with other pathogens such as influenza and RSV. High recovery rates were obtained in experiments with spiked serum samples, demonstrating the usability of the sensor in clinical samples (22). The SWV method was used in a biosensor developed for the detection of HER-2, a breast cancer biomarker. The biosensor was built on a nanohybrid-based electrode consisting of zinc oxide tetrapod (ZnOT) structures and potassium perylene tetracarboxylate (K4PTC) compound. ZnOT provides high surface area and electron conductivity, while the carboxylate groups on K4PTC allow stable binding of aptamers. The developed sensor has a linear response between 1 fg/mL and 10 μg/mL and an extremely low detection limit of 0.58 fg/mL (23). In another study conducted in 2025, a hydrogel-protected electrochemical aptamer-based biosensor (HP-EAB) was developed that can directly detect doxorubicin (DOX), a cancer drug, in whole blood samples. The aptamer, which is attached to the gold electrode with a thiol-modified tip and labeled with methylene blue (MB), changes conformation when bound to DOX, reducing the distance between the electrode and MB, which causes a significant increase in the reduction current measured by SWV. The thin agarose gel coating applied on the electrode surface prevents biofouling and increases the stability of the sensor by allowing the passage of small molecules while preventing large biomolecules (e.g. hemoglobin, albumin) from reaching the electrode. The results show that the sensor has a linear response between 0.1–50 μM and offers a detection limit of 25.9 nM. The modified form of the aptamer triggers the signaling mechanism by undergoing a structural transformation after target-specific binding, thus providing high selectivity and sensitivity (24). In a dual-electrode SWV-based electrochemical aptasensor study for the simultaneous detection of two antibiotics of high environmental importance, lincomycin and neomycin, the sensor surface was coated with a nanocomposite consisting of gold nanoparticles and carbon nanofibers providing high surface area and conductivity, and thiol-modified aptamers specifically designed for each antibiotic were immobilized. This binding mechanism of aptamers, which are immobilized on the surface by covalently binding to gold nanoparticles via thiol groups and prevent electron transfer with redox couples when the

target is bound via negatively charged phosphate backbones, causing signal decrease, made it possible to work with high selectivity and low pg/mL detection limits thanks to electrostatic interactions and three-dimensional structural specificity of the aptamer. The developed system has shown success with high recovery rates in tap water samples and is promising for environmental monitoring applications with its practical, sensitive, specific and multiple analysis capability (25).

Amperometric biosensors are typically designed to correlate the current generated by the reduction or oxidation of electroactive products—formed either in the presence of the analyte or via a mediator at the electrode surface—with the concentration of the analyte. The Differential Pulse Voltammetry (DPV) method is an electrochemical analysis method performed by adding short-term pulse signals to the linear potential sweep applied to the electrode. The current difference measured after each pulse gives a sensitive signal corresponding to the electrochemical activity of the analyte. This method offers high sensitivity and low detection limit for the determination of substances at low concentrations. Aptamer-based DPV and other amperometric biosensors have also been widely reported in the literature. Mu and co-workers developed a DPV-based biosensor for Adenosine Triphosphate (ATP) determination for use in the diagnosis of stomatological diseases. In the study, the approach of entrapping Pt\@Au nanoparticles with high electrocatalytic properties into a metal-organic skeleton structure (UiO-66) was adopted. Aptamer modification was carried out by conjugating ATP-specific DNA aptamers to the MOF surface, and this structure allowed electron transfer and particle collision by opening the "locked" sensor system when the target molecule was bound with ATP. This binding mechanism is based on the conformational change of the F-MOF structure upon binding of ATP to the aptamer, thus activating the electrocatalytic sites. The sensor stood out as an effective diagnostic platform for stomatological applications by exhibiting a very low lower detection limit of 0.046 pg/mL and a wide linear response range between 10 pg/mL – 50 μg/mL (26). In the design of a DPV based aptasensor developed for the detection of AFB1, aptamers were immobilized on the surface of N-doped carbon nanofibers/carbon fibers (N-CNFs/CFs) modified with gold nanoparticles (AuNPs) by thiol-gold chemistry. The gold-thiol interaction was both strong and directed, contributing to the conformational position of the aptamer. Since AFB1 is not electroactive, the Apt/AFB1 complex formed after binding limits the electron transfer and a decrease in the signal is observed; this makes the specific binding electrochemically detectable. The sensor showed a wide linear range between 10.0–10^8 pg/mL with a

LOD of 6.4 pg/mL and was effectively used in mixed samples of Chinese medicine with high recoveries (27). In a biosensor design, which is one of the examples of studies where different biosensor detection systems come together on a single analysis platform, a DNA self-assembly based, dual signal output biosensor was developed for the high sensitivity detection of thrombin. Both photoelectrochemical (PEC) analysis and DPV were used as measurement methods; thus, sensitivity and specificity were increased. The aptamer was modified by integrating it into the X-shaped structured DNA; DNA chains triggered by specific binding in the presence of thrombin ensure the binding of the GOx enzyme and the separation of the Fc-bearing DNA. This mechanism causes the photocurrent to decrease with the consumption of O_2 by GOx and the DPV signal to decrease with the decrease in the amount of Fc. Thus, thanks to the dual-mode system, highly sensitive thrombin detection was achieved with a femtomolar detection limit in the range of 10 fM - 10 nM (PEC: 6.89 fM, DPV: 5.86 fM) (28).

2.2. Optical aptasensors

Optical biosensors are systems that convert the interaction of biological recognition elements with the target analyte into optical signals. These sensors generally use optical principles such as fluorescence, absorbance, refractive index or surface plasmon resonance (SPR) to detect the presence of analytes. Optical-based approaches also exhibit high compatibility with aptamer-based designs due to their high specificity, fast response time and the possibility of labeled or label-free analysis. A schematic diagram depicting the most commonly used optical aptasensor techniques is presented in Figure 2. This diagram illustrates aptamer-functionalized platforms that interact with target molecules, along with optical detection methods such as colorimetry, FRET, SPR, and LFA used to monitor these interactions.

The Fluorescence Resonance Energy Transfer (FRET) method is one of the most widely used approaches in fluorescence-based aptasensor design. In the FRET method, aptamers undergo structural changes when bound to the target, affecting the distance between the donor and acceptor fluorophores; this makes it possible to correlate the signal change with the analyte concentration. The specific target recognition and conformational flexibility properties of aptamers provide significant advantages in FRET-based sensor design (29, 30). In an aptasensor developed for glucose detection, the FRET approach based on energy transfer between CdTe quantum dots (donor) and Cy3 dye (acceptor) was used. The specificity of the system for glucose was provided by the thiol-modified glucose aptamer, which binds to glucose with high affinity. While the aptamer was bound to CdTe QDs with the

TGA molecule, the FRET mechanism was activated by the inclusion of a short complementary sequence labeled with Cy3. When glucose was added to the system as a target molecule, it specifically bound to the thiol-glucose aptamer and disrupted the interaction with Cy3, which caused the cessation of FRET and the increase in the fluorescence signal. Thus, the binding mechanism is based on the control of FRET activity with the target-specific conformational change of the modified aptamer, and it gave successful results in human serum and urine samples with high sensitivity (LOD: 7.72 $\times$ 10^{-9} M) and selectivity (32). Wang et al. developed a turn-on FRET based aptamer biosensor for the simultaneous and sensitive detection of mycotoxins such as aflatoxin B1 (AFB1) and ochratoxin A (OTA). Dual fluorescent (Fam-DNA and Cy3-DNA) Fe_3O_4@TiO_2@Ag nanocomposites were used as the measurement method, and the fluorescent signal was reactivated in the presence of the target toxin and detection was achieved. Aptamers were conjugated with silver triangle nanoparticles (Ag TNPs) on the surface and when binding occurred, they changed their structures and detached from the surface, thus terminating FRET suppression and increasing the signal. This binding mechanism offers a unique design advantage based on the conformational transformation of aptamers. The developed system enabled the detection of AFB1 and OTA at low limits such as 0.26–0.94 ng/mL and was successfully applied in cereal/oil samples (33). Lu et al. developed a FRET-based ratio aptasensor for the determination of deoxynivalenol (DON). The measurement principle is based on the FRET mechanism, in which blue carbon dots (BCDs) are used as energy donors and graphene oxide (GO) as energy acceptors. Red carbon dots are also included as internal references in the system. The aptamer is covalently attached to BCDs via amidation to form the BCDs\@apt structure. These structures adsorb onto the GO surface via π–π interactions, while in the presence of DON, the aptamer binds specifically to the target, increases the distance between BCD and GO, and interrupts FRET; thus, the blue fluorescence increases again. The binding mechanism gains specificity due to the aptamer modification, thus the sensor exhibits high selectivity, low detection limit (14.7 pg/mL), and wide linear range (0.05–200 ng/mL) (34). A FRET-based aptamer biosensor was designed for the detection of the pesticide chlorpyrifos (CPF). The aptamer was conjugated to upconversion nanoparticles via amide bonds. The FRET mechanism relies on the overlap of the emission spectra of the energy donor, CIT-$NaYF_4$\:Yb^{3+},Tm^{3+}, and the absorption spectra of the acceptor, AuNPs. In this context, AuNPs bind to the complementary sequence of chlorpyrifos, establishing close contact. As demonstrated in this study, the use of

complementary sequences in FRET-based designs is a common strategy because they provide the sensor platform with a target-sensitive and reversible structure. In the absence of CPF, the aptamer and the complementary sequence hybridize, resulting in FRET, resulting in fluorescence quenching. However, in the presence of CPF, the aptamer binds to the target and the hybridized pair dissolves, thus decreasing FRET and reappearing fluorescence. The recovered fluorescence intensity is directly proportional to the chlorpyrifos concentration. The developed biosensor showed high sensitivity with a linear range of 0.1–200 ng/mL and a detection limit of 0.051 ng/mL (35). Aptasensors with FRET-based designs have also been developed for analytes containing relatively small and aromatic structures, including antibiotics. A recent example is a FRET aptasensor design based on DNAzyme and strand-displacement-based dual signal amplification, designed to simultaneously detect the antibiotics sarafloxacin (SAR) and enrofloxacin (ENR) in milk samples. The system triggers signal generation using Zn^{2+}-dependent DNAzyme arrays that are activated by binding to aptamers specific to the target antibiotics. The system generates strong FRET signals with a single excitation wavelength from FAM to ROX/JOE. The high selectivity of the aptamers prevents cross-reactivity, and the sensor stands out with both its specificity and low detection limits (1.95 pM for SAR and 5.01 pM for ENR) (36). He and colleagues developed an innovative aptamer-based FRET aptasensor based on a self-reproducing hybridization chain reaction for the detection of the antibiotic sulfamethoxazole (SME), which is present at low levels in human serum. Upon binding to SME, the aptamer folds into a specific conformation, releasing the initiator I strand that initiates HCR-1. This, in turn, triggers HCR-2, leading to the formation of long-branched DNA nanostructures that significantly enhance the signal through a feedback loop. A unique feature of this design is that the aptamer initiates a signal-enhancing DNA reaction after target-specific recognition. This allows the aptasensor to exhibit both high sensitivity and a wide linear range (0.5–2000 nM) with a low LOD of 0.301 nM (37). It is seen that the FRET method was used in an aptasensor developed for the determination of sulfadimethoxine (SDM), another sulfonamide group antibiotic. The FRET method was used in an aptasensor developed for the determination of sulfadimethoxine, another sulfonamide antibiotic. In this study, an aptamer-based FRET aptasensor was developed using a ZrFe bimetallic metal-organic framework (MOF) and ferrofluids. When the aptamer specifically binds to the target molecule, SDM, it reduces the MOF's peroxidase-like activity by allowing the complementary sequence to bind to the MOF surface. This results in a

decrease in DAP production and, consequently, FRET-induced quenching, while also increasing MOF fluorescence. The aptamer's binding to the target, the MOF's catalytic activity, and the FRET mechanism lead to measurable changes in the fluorescence signal, enabling the determination of SDM (38). While energy transfer in FRET-based methods occurs depending on the distance between two fluorophores, the fluorescence method used in some studies relies on the direct interaction of the fluorogenic aptamer with the fluorophore after target binding. In one study exemplifying this approach, Grob and colleagues developed an RNA aptamer-based fluorescent biosensor operating in *E. coli* to monitor intracellular burden. Eight different aptamers were tested in two different *E. coli* strains with various promoters, and the tRNA-Broc aptamer emerged as the most prominent, providing a high context-independent signal. However, due to their complex structures, some of these fluorogenic aptamers negatively impacted bacterial growth and created additional cellular burden. Consequently, the tRNA-Broc aptamer was identified as the most suitable biosensor, offering both low cellular efficacy and high responsiveness, and was shown to be capable of distinguishing burdens caused by different genetic constructs (39). In a 2025 study by Wang et al., an aptamer-based turn-on fluorescent biosensor was developed to image the SIRT2 protein in living cells and screen for its modulators. Twelve aptamers with high affinity for SIRT2 were selected using the magnetic bead-based SELEX (MB-SELEX) method; Aptamer 45, with the highest binding capacity, was used as the sensor's recognition element. The fluorescence measurement principle is based on the interaction of the FAM-labeled aptamer with BHQ1, a quencher, quenching fluorescence. Upon binding to SIRT2, BHQ1 is displaced, reactivating the fluorescence ("turn-on"). This system, delivered via Au nanoparticles, enabled the in vivo visualization of SIRT2 in three different cell types, and three modulators capable of increasing SIRT2 levels were identified. This approach will also allow the screening of other protein modulators by replacing the aptamer with the appropriate target protein (40). Fluorescence-based aptasensors are used to detect nucleic acid-based analytes such as RNA. In the study by Yang et al., a novel fluorescent aptamer biosensor based on ring-opening polymerization (NCA ROP) was developed for the detection of Tobacco Mosaic Virus (TMV) RNA. In the biosensor, aptamers with sequences specific to TMV RNA were immobilized on magnetic beads to enable recognition of the target RNA. Subsequently, a second aptamer (probe 2) modified with NCA-derived fluorescent peptides was hybridized with the target to amplify the signal. This fluorescence method produced fluorescence signals that increased depending on the amount of target RNA,

enabling target detection at concentrations as low as 0.085 pM (41). In a study developed by Zhang and Zhao, a fast and sensitive fluorescence biosensor based on a short DNA aptamer labeled with double-end pyrene was designed for the detection of chloramphenicol (CAP). When the CAP target molecule binds to the aptamer, it causes it to fold into a hairpin structure, causing two pyrene molecules to approach each other and form a pyrene excimer. As a result of excimer formation, a characteristic fluorescence signal was observed at 485 nm. This structure-modification-based mechanism enabled CAP determination with low background signal and high selectivity, with a detection limit of 24.4 nmol/L. The aptamer used in this study was selected using the Capture-SELEX method, and the final structure was optimized by modifying both ends with pyrene molecules (42). Wu and colleagues developed a magnetically driven dual-channel fluorescent biosensor (MDDCF) for the simultaneous detection of breast cancer-specific exosomal proteins (MUC1 and HER2). The biosensor's unique feature lies in the integration of HER2 and MUC1 aptamers with logic gates; this structure generates a signal output dependent on the recognition of both targets. The fluorescent signal is enhanced by plasmon-enhanced fluorescence (PEF) generated by the interaction of dye-labeled short sequences released after the aptamer-target interaction with silica-coated silver nanoparticles (SiO_2@Ag NPs). Unlike other designs, this system enables highly sensitive and spectrally interference-free multi-target measurement by integrating both magnetic enhancement and a dual aptamer logic gate, increasing its potential for clinical application, particularly for molecular subtype discrimination (43). Aptamer-based fluorescence sensor designs significantly increase the sensitivity and selectivity of various fluorescence mechanisms, particularly FRET, thanks to their high target-specific binding affinity and structure-modifying capabilities. The recent studies above demonstrate that the modifiable structures of aptamers offer flexible design possibilities that allow for the simultaneous and reliable measurement of both single and multiple targets. These properties provide strong advantages in fluorescence-based biosensors in terms of specificity, low detection limit, and applicability to biological samples.

While fluorescence-based aptasensors attract attention with their high sensitivity, another powerful technique that does not require labels and offers the advantage of real-time analysis is surface plasmon resonance (SPR)-based aptasensors. Santa and colleagues developed a split-aptamer-based SPR biosensor for real-time monitoring of low-molecular-weight drugs such as vancomycin. To address the signal weakness of traditional single-aptamer approaches, the aptamer was split into two fragments, one

immobilized on the surface and the other released into solution to form a ternary complex in the presence of vancomycin. This design, a notable example of the use of split aptamers, not only increased the SPR signal approximately 10-fold but also ensured the sensor's reversibility and stability throughout its exposure time. Furthermore, this sensor was tested in biological samples under various pH and ion conditions, demonstrating its functionality in biological environments. Using a split-aptamer offers reversible and continuous monitoring capabilities, whereas conventional SPR systems typically operate with a full aptamer structure and more limited reversibility. However, overstabilization of split-aptamer pairs can lead to issues such as increased background signal and slow sensor resetting. Additionally, signal attenuation due to surface biofouling is a potential risk during long-term measurements (44). An aptamer-based, highly sensitive optical SPR sensor was developed for the label-free and selective detection of berberine hydrochloride, a plant-derived alkaloid. A gold film was coated on the surface of an MSM (multimode–singlemode–multimode) optical fiber, and thiol-modified aptamers were immobilized via covalent Au–S bonds. To increase sensitivity, the surface was modified with gold nanoparticles (AuNPs), which amplified the electromagnetic field via local surface plasmon resonance (LSPR). Thus, the sensor operated in the range of 0.1–500 μM, and the LOQ was reduced to 0.0467 μM with the inclusion of AuNPs. This biosensor offers potential for portability and system integration because it offers a fiber-based platform with a smaller volume compared to conventional SPR sensors. Additionally, surface functionalization with AuNPs increased the electromagnetic field intensity, thus enhancing the SPR signal. However, due to the high precision required for fiber assembly, coating, and functionalization, the fabrication process is complex and can reduce reproducibility. Compared to existing SPR sensors, this approach offers higher sensitivity and selectivity, but improvements in automation and cost-effectiveness are necessary for widespread use in clinical applications (45). In recent years, significant advances have been made in the structural and optical components of SPR biosensors. In addition to traditional gold and silver surfaces, the use of alternative plasmonic materials such as 2D nanomaterials, metal nitrides, and carbon-based structures, which enable precise control of the refractive index, is increasing. Furthermore, miniaturized optical fiber-based and waveguide systems are improving portability and multiplex analysis capabilities. This trend is supporting the use of SPR technology, which typically relies on relatively sophisticated instrumentation, in laboratory settings and beyond. Alternatives are being proposed to overcome the limitations imposed by antibodies, such

as stability, difficulty of production, and cost, through the integration of aptamers onto the SPR sensor surface. Artificial intelligence-assisted data processing algorithms are making SPR systems more suitable for multiple target analysis and high-throughput screening, and it is anticipated that these approaches will increase in the near future (46).

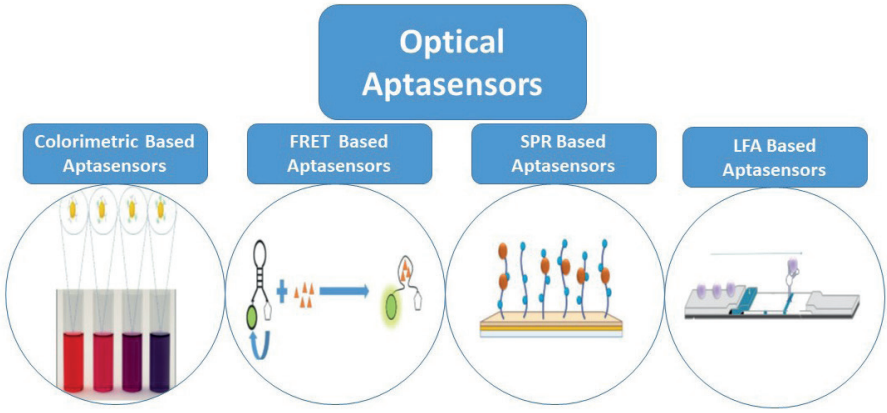

Figure 2. Schematic representation of optical aptasensors. Target molecules bind to aptamer-functionalized platforms, and the resulting signal is detected using optical techniques such as colorimetry, Fluorescence resonance energy transfer (FRET), surface plasmon resonance (SPR), and lateral flow assay (LFA).

Colorimetric biosensors offer rapid and practical analysis thanks to their simple readout systems and low-cost hardware requirements. Integrating these platforms with aptamers, thanks to their unique binding capacity and easily chemically modifiable structures, both increases selectivity and enables the development of user-friendly designs. In the study by Wei et al., a colorimetric biosensor was developed for the detection of synthetic His-tagged proteins and peptides using split aptamers as specific recognition elements and mannan oligosaccharide-coated positively charged gold nanoparticles (AuNPs-MOS) as signal transduction elements. A unique design strategy was employed to obtain split aptamers with high affinity, and a dissociation constant of 132 nM was achieved with the 6H7-Sp1/Sp2 aptamer pair derived from binding site-enriched sequences. The AuNPs, whose nanozyme activity was controlledly attenuated by MOS, produced a color change in the presence of the target molecule, enabling visual and quantitative detection. The developed biosensor demonstrated successful performance in terms of both selectivity and sensitivity, and its applicability to real bioprocess samples was confirmed (47). FRET-based systems have

also been frequently used in viral analyses, including HIV detection (31, 48). A dual-mode RNA-splitting aptamer biosensor targeting the Tat peptide was developed for the early diagnosis of HIV infection. The biosensor combines both colorimetric and fluorescent detection modes using gold nanoparticles (AuNPs) and FAM-labeled RNA aptamers. In the presence of the Tat peptide, the aptamers bind to the target and prevent its adsorption to the AuNPs surface, triggering ThT-mediated aggregation, resulting in a color change and recovery of the fluorescent signal due to the removal of the FRET effect. The developed method provided a low detection limit of 0.28 nM and a wide linear range between 0.5 and 60 nM, enabling successful measurement in human serum. Thus, the study demonstrates a low-cost, practical, and convenient platform for early diagnosis that combines both sensitivity and visual agility (48). Liu and colleagues developed a high-affinity aptamer (Apt-C5) capable of simultaneously recognizing *Cronobacter sakazakii*, *C. malonaticus*, and *C. turicensis*, thus designing an innovative biosensor to address a significant public health problem. The novelty of the study was the successful application of a multiple target selection strategy, Multiple Bacteria Alternate-SELEX (MBA-SELEX), resulting in an aptamer with cross-recognition against three different Cronobacter species. The resulting aptamer was shown to bind to lipopolysaccharides with high specificity, demonstrating that the interaction mechanism relies on the outer membrane structure. The detection mechanism is based on the color change principle, which is based on the binding-destabilization relationship of the aptamer with the peroxidase-like activity of Pd/Au biconic nanozymes. When the aptamer binds to the nanozyme, catalytic activity is suppressed. In the presence of target bacteria, the aptamer binds to the bacteria and dissociates from the nanozyme surface, resulting in the recovery of the color signal. With this strategy, the detection limit for each of the three pathogens was determined to be in the range of 15–26 CFU/mL, and successful detections were achieved in infant formula. This study makes a significant contribution to the literature regarding both the aptamer selection process and nanomaterial integration, while also highlighting the need for more extensive field validation (49). A study published by Chang and colleagues in 2025 developed an aptamer-based microneedle biosensor platform aimed at early assessment of cardiovascular disease risk by measuring C-reactive protein (CRP) levels. In the study, microneedle arrays, manufactured from polylactic acid and functionalized with CRP-specific aptamers via gold coating, were designed to capture biomarkers directly from the skin or blood. The sensing mechanism begins with the binding of the target protein to the aptamer at the microneedle tip, followed by a color (TMB-

based) colorimetric analysis using primary and HRP-conjugated secondary antibodies, providing the user with visible detection. The developed system enabled CRP determination in the range of 0–10 mg/L and was specifically designed to consider the clinical significance of levels above 3 mg/L in terms of cardiovascular disease risk. Unlike similar microneedle-based sensors in the literature, this study is both disposable and suitable for home use due to its portability and low cost. Furthermore, aptamers are more stable and reproducible than antibodies, making the system more reliable. However, the sensor design still has some limitations; for example, skin penetration depth can be affected by individual differences, and because the system does not establish a definitive correlation between CRP in interstitial fluid and blood levels, its clinical validity is not yet fully established. Nevertheless, the study demonstrates that the aptamer-microneedle combination is a powerful and innovative approach for field diagnostic applications (50). Colorimetric aptamer-based sensors are platforms that provide practical and cost-effective detection of analytes both qualitatively and quantitatively with advanced techniques through color changes visible even to the naked eye. These designs are often integrated with optically active nanomaterials, such as gold nanoparticles, and utilize the principle of aggregation or dissociation due to target-aptamer interaction. Specific aptamer binding to the target alters the stabilization equilibrium on the nanomaterial surface, resulting in a color change. Such systems reduce equipment dependency and offer user-friendly solutions for rapid field screening. At the same time, aptamer design, surface chemistry, and analysis environment conditions must be carefully optimized to achieve low detection limits.

In optical approaches based on lateral flow assays (LFA), there are biosensor studies that utilize both traditional labels such as AuNPs and innovative methods such as fluorescence-based techniques. In a study published by Wang et al. in 2025, an aptamer-based LFA platform was developed for the rapid and sensitive detection of AFB1, one of the most potent mycotoxins that threaten food safety, in different agricultural products. Eight aptamers identified in the literature were compared in detail using molecular dynamics (MD) simulations and the SYBR Green I fluorescence assay. Aptamer 7, with the highest binding energy and structural stability, was selected for further studies. The detection mechanism is based on AFB1 competing with the target molecule, preventing the interaction of the gold nanoparticle (AuNP)-supported aptamer with the capture probe on the test line. Thus, the red color intensity on the test line decreases with increasing AFB1 concentrations. This competitive sensor design exhibited a linear response between 1–200 ng/mL and a very low detection limit of

0.23 ng/mL. A notable aspect of the study is that the aptamer selection was guided by detailed MD-based analyses prior to the experimental steps. This approach provides a rational and cost-effective strategy for sensor design (51). In one study that exemplifies the creative use of aptamer-based systems in the literature, an "indirect lateral flow" approach was adopted, using a commercial LFA kit to detect not the target analyte directly, but a molecule (morphine) released upon interaction with it. The detection mechanism relies on the dissociation of the capping aptamer from mesoporous silica nanoparticles capped with an aminoglycoside aptamer upon encountering the target antibiotic, kanamycin, thereby releasing the loaded morphine molecules. The released morphine is visually detected by a commercial MOP LFA assay, indirectly detecting the presence of kanamycin. A notable aspect of this approach is the use of the aptamer without any marker modification and the integration of commercially available kits for signal generation, rather than designing a new LFA kit. This novel design is particularly promising for low-cost and portable diagnostic systems; however, controlling potential false-positive sources such as morphine may require additional specificity assessments. Additionally, the need for interaction time as long as 4 hours may be a limiting factor for real-time POCT applications (52). The aptamer-based fluorescent LFA biosensor, developed for the detection of *Staphylococcus aureus* (*S. aureus*), goes beyond traditional color-change-based assays and generates a signal based on the binding of a Cy5-labeled aptamer to the target. The interaction mechanism is based on specific binding competition between the target bacteria and the aptamer; in the presence of the target, the aptamer binds to the target and displaces the complementary sequence, resulting in a decrease in the fluorescence signal in the test line. The system, which boasts high specificity and a low detection limit (1.65 CFU/mL), has been successfully tested in both milk and chicken meat samples. However, due to its dependence on fluorescence readers and the complex optimization process, the method requires additional engineering solutions for widespread use in field applications (53). A study conducted for cadmium detection presents a striking innovation in the design of an aptamer-based LFA developed for the detection of small molecules, with its signal-on mechanism. Unlike traditional signal-off approaches, the formation of a signal in the presence of Cd^{2+} simplifies visual interpretation and prevents false negatives. Small molecules often struggle to generate sufficient signal with aptamers due to their limited epitope surface area and low molecular weight. This problem was overcome by utilizing the principle that the aptamer undergoes a conformational change upon binding to Cd^{2+}, preventing hybridization. In the presence of Cd^{2+}, the aptamer forms a hairpin-like structure, closing the

binding site, allowing the AuNP conjugates to reach the test line, generating a positive signal. This results in both increased specificity and a system that can be easily assessed with the naked eye, providing a highly useful and practical solution for environmental monitoring applications (54). Table 1 shows some parameters of electrochemical and optical based aptasensors.

Table 1. Current electrochemical and optical based aptasensors based on different methods and some of their properties.

Targets	Detection method	Aptamer modification method	Linear range	Reference
PTH fragment	EIS	5′-NH_2	20 to 600 pg/mL	14
EGFR	EIS	Unlabeled	10 fg/mL to 100 ng/mL	15
TRA	EIS	5′-NH_2	50 pM to 1.3 nM	16
AFB 1	EC/EIS	5′-SH and 3′- FC	10 pg/mL and 30 ng/mL in ratiometric EC mode and between 3 pg/mL and 30 ng/mL in EIS	17
Escherichia coli	EIS	5′-NH_2	100–10^4 CFU/mL	18
CHIKV	EIS	Unlabeled	1 ng/mL to 10 μg/mL	19
P21 protein	EIS	5′-SH	0.47 – 1.88 μg/mL	20
11-DCL	SWV	5′-SH	0.01 – 100 pg/mL	21
SAG1	SWV	Unlabeled	0.01–100 nM	22
HER2	SWV	Unlabeled	1 fg/mL – 10 μg/mL	23
DOX	SWV	5′-SH and 3′-MB	0.1–50 μM	24
Lincomycin and Neomycin	SWV	5′-SH	Lincomycin: 0.02 pg/mL Neomycin: 0.035 pg/mL	25
ATP	DPV	Unlabeled	10 pg/mL to 50 μg/mL	26
AFB 1	DPV	5′-SH	10.0–10^8 pg/mL	27
Thrombin	PEC and DPV	Unlabeled	10 fM – 10 nM	28
Glucose	FRET	5′-SH	0.1 μM – 1.0 μM	32
AFB 1 and OTA	FRET	Both aptamers are unlabeled, other DNAs are FAM and Cy3 labeled	AFB 1 25–250 ng/mL OTA 0–100 ng/mL	33
DON	FRET	5′-COOH	0.05–200 ng/mL	34
CPF	FRET	5′-NH_2	0.1–200 ng/mL	35
SAR and ENR	FRET	Unlabeled	25 pM – 100 nM	36

SME	FRET	Unlabeled	0.5–2000 nM	37
SDM	FRET	5′-NH_2	10 pM – 10 nM	38
Cellular burden in *E. coli*	Fluorescence via fluorogenic RNA aptamers	Unlabeled	Not defined	39
SIRT2	Fluorescence "turn on"	5'-FAM and 3'-BHQ1	0.1–5 μg/mL	40
Tobacco Mosaic Virus RNA	Fluorescence	5′-NH_2	pM – 10 nM	41
CAP	Fluorescence	5′ and 3′ pyrene	24.4 nmol/L – 50 μmol/L	42
Exosomal MUC1 and HER2	Plasmon-amplified dual-channel fluorescence	Unlabeled	10^2–10^7 particles/μL	43
Vancomycin	SPR	Split-aptamer	0.05–100 μM	44
Berberine hydrochloride	SPR	5′-SH	0.1 – 500 μM	45
His-tag, His-tagged proteins, His-tagged peptides	Colorimetric assay	Split-aptamer	14.60 – 3750 nM (for 6×His-tag)	47
HIV Tat peptide	Dual-mode – colorimetric assay (UV-Vis) and fluorescence spectroscopy	3'-FAM	For the fluorescence method: 0.5–25 nM and for the colorimetric method: 2–60 nM	48
Cronobacter sakazakii, Cronobacter malonaticus, Cronobacter turicensis	Nanozyme-based colorimetric assay	5'-FAM and Unlabeled	10^2 – 10^8 CFU/mL (for all three *Cronobacter* species and mixtures)	49
CRP	Colorimetric assay	5'-SH and 5'-SH/3'-FITC dual-labeled ssDNA	0–10 mg/L	50
AFB 1	LFA	Unlabeled	1–200 ng/mL	51
Kanamycin	LFA	Unlabeled	10 nM to 350 nM and 500 nM–1500 nM	52
S. aureus	LFA	5'-Cy5	2.8×10^1 to 2.8×10^8 CFU/mL	53
Cadmium ion	LFA	5'-SH	1.0–5000 ppb	54

3. Conclusion

This chapter presents an analysis of the performance parameters of current electrochemical and optical approaches using nucleic acid aptamers as recognition elements. Current methodologies are assessed, encompassing the benefits and drawbacks of aptamers in comparison to conventional recognition elements. The significant rise in the utilization of aptamers in biosensor designs, particularly during the past five years, is undeniable. This document provides a detailed explanation of the labeling and application of aptamers to biosensor surfaces. Additionally, the evaluation of novel design strategies that can enhance field application has been conducted, specifically for biosensors developed in the past year. This evaluation clearly demonstrates the potential offered by aptamer-based electrochemical and optical biosensors in terms of performance criteria such as selectivity, sensitivity, stability, and portability. Electrochemical approaches stand out with their low-cost hardware and miniaturization capabilities, while optical methods offer high sensitivity, particularly in fluorescence-based systems. The ability to chemically modify aptamers has enabled the development of platforms that enable multiple target detection. However, several technical challenges, such as matrix effects, surface modifications, and integration with portable devices, remain unresolved. Future work will focus on the development of low-cost, high-accuracy systems that increase field usability and accelerate the integration of these technologies into real-time and widespread diagnostic applications.

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