CHAPTER SIX

Advances in aptasensor technology

Laleh Hosseinzadeh[a], **Mohammad Mazloum-Ardakani**[b,*]
[a]Department of Chemistry, Dehloran Branch, Islamic Azad University, Dehloran, Iran
[b]Department of Chemistry, Faculty of Science, Yazd University, Yazd, Iran
[*]Corresponding authors: e-mail addresses: mazloum@yazd.ac.ir; mazloumardakani@gmail.com

Contents

Abstract

Aptasensors form a class of biosensors that function on the basis of a biological recognition. An aptasensor is advantageous because it incorporates a unique biologic recognition element, i.e., an aptamer, coupled to a transducer to convert a biological interaction to readable signals that can be easily processed and reported. In such biosensors, the specificity of aptamers is comparable to and sometimes even better than that of antibodies. Using the SELEX technique, aptamers with high specificity and affinity to various targets can be isolated from large pools of different oligonucleotides. Nowadays, new modifications of the SELEX technique and, as a result, easy generation and synthesis of aptamers have led to the wide application of these materials as

Advances in Clinical Chemistry, Volume 99
ISSN 0065-2423
https://doi.org/10.1016/bs.acc.2020.02.010

biological receptors in biosensors. In this regard, aptamers promise a bright future. In the present research a brief account is initially provided of the recent developments in aptasensors for various targets. Then, immobilization methods, design strategies, current limitations and future directions are discussed for aptasensors.

1. Introduction

The detection and identification of pathogenic agents are very important in clinical diagnostics and biomedical research. Because the concentration of pathogenic agents in human fluids, such as urine and blood, is very low, it is imperative to develop selective and sensitive methods of detection. This approach allows for earlier diagnosis and more effective treatment of diseases [1,2]. In comparison with conventional techniques, aptamer-based biosensors have considerable advantages in the field of clinical analysis due to the unique properties of aptamers as biological recognition elements. In this respect, one can refer to such properties as high specificity and affinity to targets, small size, easy synthesis, high stability even in non-physiological conditions, simple chemical modification, and resistance to degradation and denaturation [3–5]. Therefore, aptasensors can compensate for the limitations of current clinical diagnostics.

Biosensors provide for rapid analysis and real-time detection. They are made up of two main components including a transducer and a biological recognition element. An aptasensor is a particular type of biosensor in which an aptamer serves as a biological recognition element. This element is a biomolecule that binds to a specific target analyte. The transducer converts this bond to detectable and measurable signals. There are various transducers such as electrical, optical and mass-sensitive ones [6–8].

In this chapter, the current knowledge of aptasensors is reviewed for researchers who are interested in designing and developing aptasensors. Some examples are also provided of the recent progress in aptasensors. In this case, references are made to recently published research conducted particularly on the potential and actual applications of aptasensors in clinical diagnostics. In addition, an overview is given of the common immobilization techniques, the existing design strategies to construct aptasensors, and the target analytes that have been measured until now. Finally, the limitations and strengths of these sensors as well as their future challenges and applications are discussed.

2. Aptamer-based biosensors: Aptasensor

Aptamers are short (20–60 nucleotides) single-stranded oligonucleotides (RNA or DNA) that are capable of binding various targets such as proteins, peptides, carbohydrates, small molecules, metal ions, toxins, and even live cells with high specificity and affinity [9–15]. The term "aptamer" was coined by Ellington and Szostak [16]. Aptamers can be obtained from the compounds in the very large class of oligonucleotides by an in vitro selection process called systematic evolution of ligands by exponential enrichment (SELEX). The SELEX process can be separated into two alternating steps (Fig. 1). At the first step, the original oligonucleotides are amplified through a polymerase chain reaction to the desired concentration. At the second step, the amplified pool is incubated with certain targets, and interacting oligonucleotides are used for the first step of the next SELEX round. Using this technique, aptamers with high specificity and affinity to their target molecules can be isolated from large pools of different oligonucleotides in a highly iterative way. In this case, the oligonucleotides which bind to a target are eluted and amplified by a polymerase chain reaction, while the unbound ones are washed away [17–21].

Biosensors are made up of two main components including a transducer and a biological recognition element. The biological element is a biomolecule

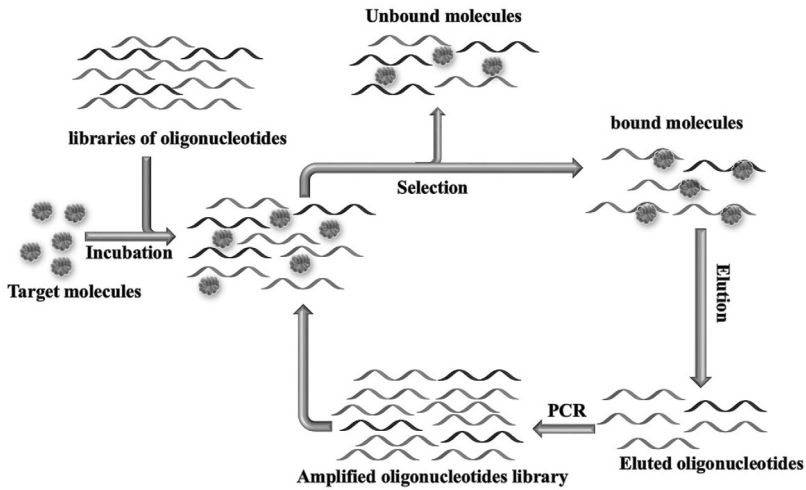

Fig. 1 Systematic evolution of ligands by exponential enrichment (SELEX) process.

(e.g., antibodies, aptamers, proteins or peptides) that can bind specifically to the respective target. An aptasensor is a particular type of biosensor in which the biological recognition element is an aptamer. It is worth noting that aptamers have many advantages over other biological recognition elements such as antibodies. This is due to the remarkable advantages of aptamers such as ease of synthesis, inexpensiveness, high specificity and affinity to various target molecules, high chemical stability in experimental conditions, and easy chemical modification with different reporter tags. Owing to the unique properties of aptamers, aptasensors play important roles in clinical diagnostics, disease monitoring, biomarker identification and the analysis of other samples of various types [22–26].

3. Immobilization

The first essential step in developing an aptasensor is the immobilization of an appropriate aptamer on a conducting surface such as metal, carbon nanotube or polymer [27–29]. Recently, various immobilization strategies have been developed. Aptamers can be immobilized directly on the surface of a sensor or indirectly via chemical coupling. The direct immobilization of aptamers is mainly the result of electrostatic interactions, hydrogen bonds or van der Waals forces. For the immobilization of aptamers through chemical coupling, however, different strategies can be used. The variety of applicable strategies in this case suggests the easy modification of aptamers [30]. The choice of an immobilization technique depends on several factors, such as the nature of the biological recognition element and the transducer surface, the properties of the target molecule and the operating conditions of the aptasensor [31]. Here, an overview is provided of several common methods for the immobilization of aptamers on different substrates, including the use of gold-sulfur self-assembled monolayers, covalent bonds, biotin/avidin affinity, complementary nucleic acid chain connections and the novel methods reported recently.

3.1 Direct attachment to the gold surface

In this method, an aptamer is modified with a thiol group (–SH). The direct immobilization of the thiolated aptamer forms a stable and flexible self-assembled monolayer of the aptamer on the gold surface through a gold-sulfur interaction [32]. The formation of bonds between sulfur and gold are due to the strong affinity between the thiol group and the gold surface

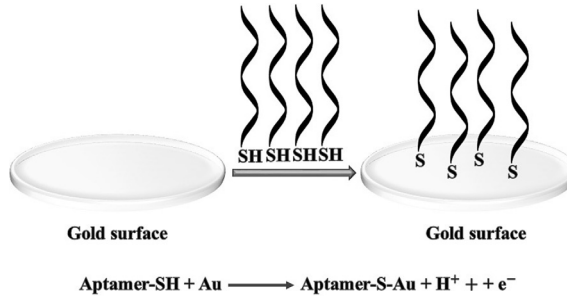

Fig. 2 Schematic of aptamer immobilization on the gold surface.

[6,31,33]. The aptamer is immobilized on the gold surface via the 5′-end or the 3′-end. The 3′-end is more suitable because it creates resistance to nuclease after its binding to the electrode surface. Direct attachment aptamer to gold surface was reported in 2002 for the first time [34]. The immobilization of aptamer on gold surface is shown in Fig. 2.

In this method, i.e., the direct attachment of the aptamer to the gold surface through a gold-sulfur interaction, the gold surface can be an electrochemical gold electrode [35,36], a surface plasmon resonance (SPR) sensor [37,38], a quartz crystal microbalance (QCM) sensor [39,40], or other gold-coated surfaces such as gold nanoparticles [41,42] and gold nanorods [43,44].

In 2019, Arduini et al. [45] successfully designed an aptasensor by a two-step method in which a thiol-modified aptamer was self-assembled on a gold screen-printed electrode surface through a gold-sulfur interaction. At the first step, a gold screen-printed electrode was functionalized by the placement of an aptamer solution on the surface of a gold working electrode. Then, the electrode was incubated at 4 °C for 2 days. Subsequently, the aptamer-modified gold screen-printed electrode was washed with distilled water to eliminate non-specific physical adsorption. Following the incubation, 6-mercaptohexanol in a Dulbecco buffer solution was added onto the electrode to remove nonspecifically adsorbed aptamer molecules and to passivate the electrode surface. The as-prepared aptasensor was used for the detection of *B. cereus* spore. In 2013, Săndulescu et al. [46] successfully constructed two simple electrochemical aptasensors for the detection of the MUC1 tumor marker. The recognition of the marker was done with thiolated aptamers immobilized on a gold nanoparticle-modified graphite screen-printed electrode and a gold nanoparticle-modified graphite gold screen-printed electrode. The quantitative detection of MUC1

protein took place by electrochemical impedance spectroscopy (EIS) and differential pulse voltammetry (DPV). Calibration curves were obtained with both techniques under optimized conditions. The detection limit of the MUC1 protein was estimated to be $3.6\,ng\,mL^{-1}$ at the gold nanoparticle-modified graphite screen-printed electrodes by EIS and $0.95\,ng\,mL^{-1}$ at the gold nanoparticle-modified gold screen-printed electrodes by DPV. The comparison of the two calibration curves indicated that the electrochemical method in which an aptamer probe is immobilized on gold nanoparticles is more convenient and allows the quantitative detection of MUC1 protein. In 2019, Blidar et al. [47] developed a label-free SPR aptasensor for AMP detection using an enhanced thiolated aptamer immobilized on a gold chip through the potential pulsing electrochemical method. Each step of the aptasensor design was characterized using a hybrid electrochemical-optical procedure and through SPR and electrochemical analyses. The efficiency of the interaction between the immobilized aptamer and AMP was estimated by the QCM analysis. This aptasensor was able to detect AMP with a LOD of $1\,\mu mol\,L^{-1}$. The selectivity of the aptasensor was also tested in the presence of other antibiotics and drugs.

3.2 Covalent attachment method

Aptamers can covalently be attached to chemically modified surfaces. In this method, the surface of a sensor is modified by a chemical functional group, such as amine: $-NH_2$, hydroxyl: –OH, or carboxylic acid: –COOH. This functional group interacts with a modified aptamer which has a corresponding chemical functional group. Thus, an aptamer layer is formed on the sensor surfaces. Some of these interactions are shown in Fig. 3.

In 2005, Lee et al. [48] successfully developed a single-walled carbon nanotube field-effect transistor aptasensor using an aptamer as an alternative to protein-based sensing elements. In their work, the thrombin aptamer was modified with an amine group for immobilization purposes. Basically, as also occurred in that study, N, N′-carbonyldiimidazole (CDI) reacts with hydroxyl groups of tween 20 to form an active imidazole carbamate intermediate. In the presence of an aptamer modified with the $-NH_2$ group, the active imidazole carbamate in CDI-Tween reacts with the NH_2 group, and a carbamate linkage is formed at the cost of imidazole (Fig. 3A). To block any unreacted surface groups that can further react with other proteins, the sample is incubated in an ethanolamine solution.

In 2013, Jang et al. [11] fabricated a flexible liquid-ion gated field-effect transistor-type graphene aptasensor for Hg. Graphene nanosheets were

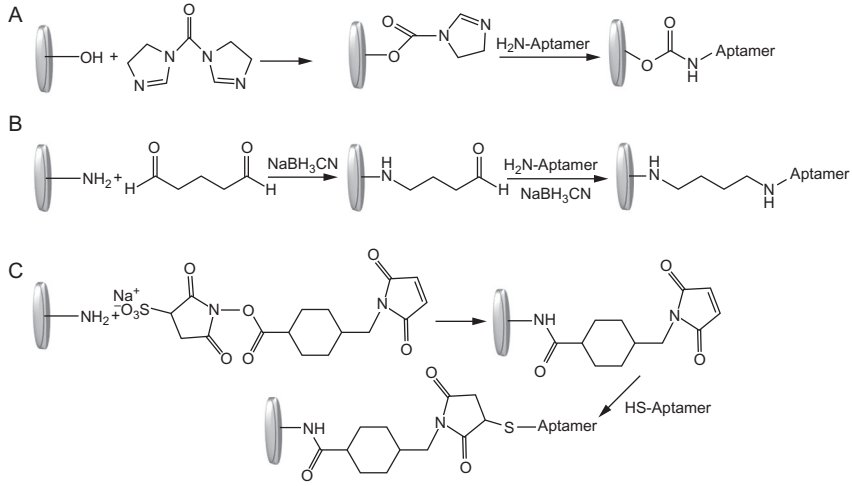

Fig. 3 (A, B, C) Common reaction reported for covalent attachment of aptamers to surfaces.

chemically modified using 1,5-diaminonaphthalene (DAN) through a π-π interaction between the phenyl groups of DAN and the graphene sheets. The aptasensor was, indeed, fabricated by the immobilization of an aptamer modified with the $-NH_2$ group after the addition of glutaraldehyde (GA). During the chemical reactions, one aldehyde group of GA was connected to the $-NH_2$ group of DAN through chemical bonding. Similarly, the other aldehyde group of GA was bonded to the $-NH_2$ group of the aptamer, resulting in an aptamer immobilized on the graphene surface. DAN and GA were used as efficient cross-linking agents to immobilize the aptamer on the graphene surface (Fig. 3B). In 2016, Demirkol and Timur [49] used quantum dot/aptamer bioconjugates to design a sandwich-type aptasensor as a signal label for the analysis of *E. Coli* O157:H7 in a microtiter plate format. A thiolated aptamer was immobilized on a 96-well plate surface coated with Poly-L-lysine. It served as a support material. It was also used in a sandwich assay with quantum dots/NH_2-aptamer conjugates as a secondary aptamer. Covalent immobilization was conducted and the amino groups of poly-L-lysine were attached to the thiol groups of the aptamer via 4-(N-maleimidomethyl)cyclohexane-1-carboxylic acid 3-sulfo-N-hydroxysuccinimide (Fig. 3C). The fluorescence signals were followed before and after the addition of bioconjugates. The bioassay could detect pathogens down to 102 CFU/mL with high selectivity.

This method has such advantages as good stability, flexibility, high binding strength, the increased specificity of the aptasensors and the decreased

non-specific adsorption. On the other, the method requires complicated steps such as chemical modification and involves many annoying factors like surfactants, cross-linking agents as well as certain side effects. Therefore, the optimum reaction conditions of various factors must be taken into consideration [33,50].

3.3 Biocoatings

The specific and strong affinity between avidin (or its derivatives) and biotin can be used for the immobilization of aptamers to construct aptasensors. In this technique, the immobilization of a biotinylated aptamer on a surface coated with avidin or its derivatives can be done via specific noncovalent interactions and using biotin-avidin. Neutravidin and streptavidin are two derivatives of avidin. These proteins have a similar affinity to biotin, despite their substantial difference in terms of physical and chemical characteristics. The binding of streptavidin and neutravidin to biotin is more energetically favorable than that to avidin. This is because of the stronger interactions in the former case. In addition, neutravidin has a higher degree of non-specific adsorption than avidin and streptavidin [51]. Therefore, streptavidin is preferred over avidin and neutravidin in the immobilization of aptamers on the sensor surfaces.

In 2010, Gu et al. [52] reported a simple electrochemical aptasensor for the detection of tetracycline using a tetracycline-binding ssDNA aptamer. First, they treated a screen-printed gold electrode with 3,3′-dithiodipropionic acid to form a self-assembled monolayer on the working electrode. The carboxylic groups on the electrode were activated with of N-(3-dimethylaminopropyl)-N-ethylcarbodiimide (EDC) and N-hydroxysuccinimide (NHS). Then, streptavidin was covalently coupled to the carboxylic acid groups. Finally, a biotinylated aptamer was incubated on the streptavidin-coated electrode for binding tetracycline. In 2013, Choo et al. [53] introduced a sensitive method for the fast and reproducible detection of thrombin using the surface-enhanced Raman scattering technique and based on aptamer-conjugated magnetic beads and gold nanoparticles. First, a thiolated thrombin-binding aptamer was immobilized on the surface of gold nanoparticles. As previously stated, thiol groups have a high affinity for gold surfaces and form stable connections with them. Next, streptavidin-coated magnetic beads and a biotinylated thrombin-binding aptamer were immobilized on the surface of the magnetic beads through an avidin-biotin interaction. Finally, the sandwich aptasensor formed between the two aptamers was bound to different labels, and thrombin was

sandwiched between the two different types of aptamer-conjugated particles. In 2007, Hianik et al. [54] constructed a QCM aptasensor for the detection of human thrombin. They used two phenothiazine dyes, methylene blue (MB) and methylene green (MG), to immobilize the aptamer. The aptasensors based on the conducting polymers MB and MG were compared in terms of their ability to detect thrombin. Firstly, AT-cut quartz crystals covered by gold electrodes were cleaned and placed in an EQCM cell. Phenothiazine dyes were polymerized on the gold electrode surface. Avidin was electrostatically precipitated or cross-linked with human serum albumin (HSA) by the glutaraldehyde and biotinylated aptamers immobilized on surface through the avidin-biotin interaction. For the electrostatical precipitation, an electrode covered with poly (phenothiazine dye) was immersed in an avidin solution, and potential cycling was done. For cross-linking with HSA, an electrode was immersed in a solution obtained from avidin, HSA and glutaraldehyde in Tris buffer, and the potential was cycled. The free aldehyde groups remaining on the electrode surface were blocked with a glycine solution. The efficiency of phenothiazine polymerization and immobilization strategies was tested simultaneously by cyclic voltammetry (CV) and the EQCM technique involved in the QCM method.

3.4 Other substrates

Another method that can be used to immobilize an aptamer on the surface of a sensor is hybridization with partially complementary oligonucleotides. This immobilization strategy involves annealing and hybridization steps; therefore, the experimental conditions are difficult to control. In 2015, Zhang et al. [55] demonstrated a label-free sensitive electrochemical aptasensor for the amplified determination of lysozyme. In this work, first, the designed double-stranded DNA was immobilized on a gold electrode. One of the sequences was a 3′-terminal thiolated sequence, and the other was a lysozyme-binding aptamer. In the presence of lysozyme, the special binding of lysozyme to the lysozyme-binding aptamer with high affinity denatured the double-stranded DNA, and an aptamer/lysozyme complex was released into the solution. The thiolated sequences remaining on the surface of the electrode were applied for further hybridization chain reaction. To detect this process, two hairpin DNA probes, i.e., HP1 and HP2, were applied. Generally, HP1 contains 19 bases that are completely complementary with a thiolated sequence at 5′-terminal, and HP2 has two series of sequences complementary with HP1. Once HP1 and HP2 are

added, the hairpin structure of HP1 opens and exposes the remainder of the sequence of HP1, which is hybridized with parts of the sequence of HP2 and then opens HP2. Then, the exposed sequence of HP2 is matched with the complementary sequences in HP1. Finally, a hybridization chain reaction is triggered, which generates a complex structure of a HP2-HP1-thiolated sequence on the electrode surface. In the study conducted by Zhang and colleagues, since the cytosine-rich sequence was labeled on the 3′-end of HP2, in the presence of silver ions and $NaBH_4$, DNA/silver nanoclusters were formed on the HP2-HP1-thiolated sequence structure. Lysozyme could be detected based on the peroxidase-like character of DNA/silver nanoclusters. In 2007, Brook et al. [56] reported a simple and rapid colorimetric aptasensor. Adenosine aptamers were attached to gold nanoparticles by hybridization with complementary oligonucleotides that were immobilized on the gold nanoparticles. Afterwards, the gold nanoparticles solution turned red due to the electrostatic repulsion from much more negative charges on partially complementary double-stranded DNA. When the target molecules were added, the aptamers preferred the adenosine and were dissociated from the nanoparticles. At this stage, the nanoparticles were aggregated together and the solution turned to purple as the surface of the gold nanoparticles reduced the negative charges significantly.

In some reports, Aptamers were directly attached to gold surfaces through a gold-nitrogen interaction [57,58]. In 2008, Tok et al. [58,59] fabricated an aptamer-based surface-enhanced resonance Raman scattering (SERRS) sensor for the detection of thrombin. In this work, colloidal gold nanoparticles were aggregated on a glass surface. Then, an MB-conjugated thrombin-binding aptamer was added to the aggregated substrate of gold nanoparticles. Finally, different concentrations of thrombin were introduced, and the corresponding SERRS spectra were derived. As it is known, the immobilization of a thrombin-binding aptamer on the surface of gold nanoparticles is done only through electrostatic interactions between nitrogenous bases and the surface of those gold nanoparticles. This aptamer is susceptible to displacement from the surface. It occurs as the aptamer recognizes and binds to target molecules.

In 2007, Pelton et al. [60] reported the adsorption and covalent coupling of adenosine triphosphate (ATP)-binding aptamers onto the surface of cellulose. They investigated the physical aspects of this experiment in order to devise a procedure for applying paper surfaces to detect pathogens. In their study, the oxidation reaction on the cellulose membrane generated

aldehyde groups on that membrane. Fluorescently labeled DNA (FDNA) was immobilized on the oxidized cellulose membrane via the covalent coupling of an amine-terminated DNA aptamer with an oxidized cellulose film. Then, double-stranded DNA was formed between the FDNA and a short DNA molecule that was a 3′-terminal one labeled with a fluorescent quencher (QDNA). The quencher and the fluorophore were in close proximity. As a matter of fact, double-stranded DNA does not fluoresce; however, in the presence of ATP, a special binding of ATP to FDNA with high affinity denatures that DNA and the QDNA strand cleaves it. As this occurs, a fluorescence signal can be detected. In the study conducted by Pelton and colleagues, confocal laser microscopy was used to determine the fluorescence intensity of the cellulose membrane before the addition of QDNA, after the incubation with QDNA, and after the incubation with ATP.

In 2005, Lee et al. [48] designed a single-walled carbon nanotube field-effect transistor (SWNT-FET) aptasensor for the detection of thrombin. A thrombin-binding aptamer with a high affinity for serine protein thrombin was immobilized on the SWNT-FET aptasensor using CDI-Tween linking molecules. While the Tween component was bound to a carbon nanotube sidewall through hydrophobic interactions, the active imidazole carbamate in the CDI-Tween reacted with the NH_2 group in the 3′-amine-terminated thrombin-binding aptamer, thereby forming a carbamate linkage. The electrical transfer characteristics of the SWNT-FET sensor were measured at each stage of the process. It is to be noted that the binding of thrombin-binding aptamers on SWNT-FETs causes a rightward shift of the threshold gate voltages, presumably due to the negatively charged backbone of the DNA aptamers. While the addition of a thrombin solution causes an abrupt decrease in the conductance of the thrombin-binding aptamer immobilized on an SWNT-FET, no noticeable change is observed in the elastase.

In 2015, Zhang et al. [61] used nitrogen-doped graphene quantum dots (N-GQDs) to construct a label-free photoelectrochemical aptasensor for the detection of chloramphenicol (CAP). The aptasensor was fabricated by the immobilization of the N-GQDs and the aptamer on the surface of an FTO substrate. The large specific surface area and the π-conjugated structure of the N-GQDs ensured the facile immobilization of the aptamer on the N-GQDs through a π-π stacking interaction between the nucleobases and the N-GQDs. When CAP was present in a solution, it could be captured by the aptamer, which then reacted with the photo-generated holes on the N-GQDs to produce an enhanced photocurrent signal.

4. Design strategies

Different designed strategies may be applied in the construction of aptasensors depending on the usage envisaged for them. So far, several such strategies have been proposed on the basis of aptasensor categories. In 2009, Yu et al. [62] classified electrochemical aptasensors into three categories in terms of the change that the involved analytes induce. The changes include configurational change (i.e., the analyte binding induces either an assembly or dissociation of the sensor construct), conformational change (i.e., the analyte binding induces an alteration in the conformation of the surface-immobilized aptamer strands) and conductivity change (i.e., the analyte binding "switches on" the conductivity of the surface-bound aptamer–DNA constructs). In 2010, Zhou et al. [63] classified the strategies or modes for the fabrication and application of aptasensors into four categories including target-induced structure switching mode, sandwich or sandwich-like mode, target-induced dissociation/displacement mode and competitive replacement mode. Of course, in the literature, aptasensors have been introduced with other classifications such as labeled (using labeled aptamers) [64–68], label free (using non-labeled aptamers) [69–73], signal-on (with increased response) [74–77] and signal-off (with decreased response) [78–81] aptasensors. In the following sections, the strategies designed in two categories are explained and exemplified.

4.1 Labeled aptasensors

In this category, the biological recognition element can be labeled with a great variety of compounds. The label can be covalently attached to the aptamer, and the response of the aptasensor to the target is based on the response of the label after the formation of an aptamer-target complex. To date, various compounds such as enzymes and nanomaterials have been employed as labels [82–91]. One may refer to such enzymes as horseradish peroxidase (HRP), glucose oxidase (GOD) and alkaline phosphatase (AP). Also, a few of the nanomaterials used in this regard are quantum dots (QDs), metal-based nanomaterials, carbon-based nanomaterials and polymers.

The aptamer changes its conformation after binding with its target. On this basis, various aptasensors have been designed for various biomolecules. In some designs, the labeled aptamer changes its conformation after binding to its target, thereby altering the position of the label and the output signal. In 2010, Revzin et al. [92] developed an electrochemical DNA aptasensor

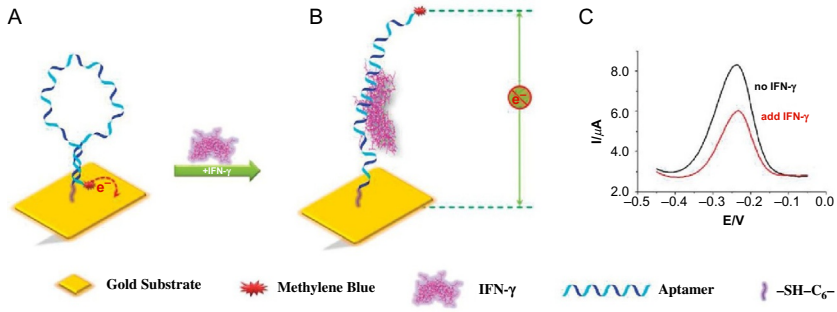

Fig. 4 (A, B) Schematic of aptamer-based electrochemical sensor for IFN-γ. (C) DPV response of aptasensor in the presence and absence of IFN-γ.

for the detection of interferon (IFN)-γ. The fabrication of this aptasensor is illustrated in Fig. 4. The direct immobilization of a thiolated aptamer at the 5′-terminal formed a self-assembled monolayer on a working gold electrode through a gold-sulfur interaction. In addition, the IFN-γ-binding aptamer was conjugated with the methylene blue (MB) label. The IFN-γ binding pushed MB label away from the electrode surface and changed the aptamer conformation. An increasing in the distance of the MB redox molecules from the electrode surface caused a decrease in the electrochemical signal.

According to the results, a better detection limit (0.06 nM) was obtained by the square wave voltammetry (SWV). This labeled "signal-off" aptasensor, proposed based on the change in the aptamer confirmation, may have broad applications in disease diagnostics and bioassay. In 2015, Roushani and Shahdost-fard [93] developed a labeled "signal-off" electrochemical aptasensor for the detection of cocaine. The aptasensor was fabricated by the covalent immobilization of a 5′-amino-3′-silver nanoparticles-functionalized aptamer on an MWCNTs/IL/Chit nanocomposite-modified glassy carbon electrode. The 5′-amino-3′-silver nanoparticles functionalized the aptamer by a terephthalate linking agent attached to the amine groups of chitosan in the nanocomposite on the electrode surface via amide coupling. Riboflavin (RF) was used as the redox probe, and silver nanoparticles accelerated the electron transfer kinetics related to the reduction of RF. Before the interaction of cocaine with the 3′-end silver nanoparticles labeled aptamer, the aptasensor could produce an electrochemical signal related to the reduction reaction of RF. After cocaine binding, the aptamer conformation changed, and the aptamer was kept near the electrode surface. Therefore, the electron transfer of RF as the probe was perturbed and a decrease occurred in the electrochemical signal. This labeled "signal-off" aptasensor, fabricated

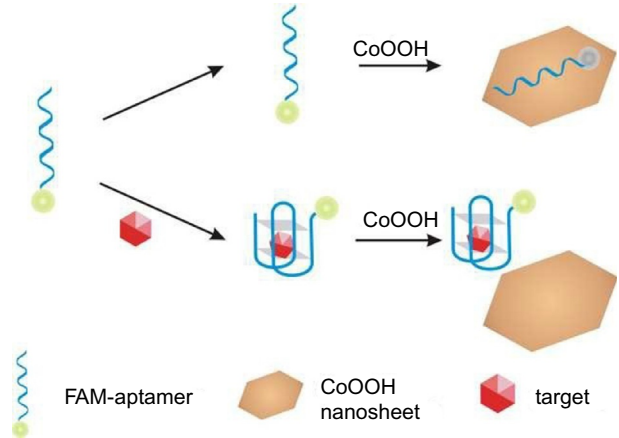

Fig. 5 Schematic illustration of designed aptasensor for the detection of thrombin.

on the basis of the change in the aptamer conformation, was successfully used to detect cocaine in human serum samples. In 2016, Chen et al. [94] reported an aptasensor for the detection of thrombin based on a fluorophore-labeled aptamer and cobalt oxyhydroxide (CoOOH) nanosheets (Fig. 5). In their study, the CoOOH nanosheets served as a quencher, and the 5′-FAM fluorescent group labeled aptamer was used as a fluorophore. The nanosheets could distinguish single-stranded DNA and G-quadruplex on the basis of their different absorption abilities. Since CoOOH nanosheets are highly capable of quenching FAM, after thrombin binding, the aptamer conformation changed and the anti-parallel G-quadruplex structure of the aptamer-thrombin complex had weaker absorption to the CoOOH nanosheets than the FAM-labeled aptamer unbound to thrombin. This strategy is significantly potential to be used in the development of other biomarkers.

Apart from the above strategy, other designs have been developed for labeled aptasensors. For example, the sandwich format is employed for the selective and sensitive monitoring of different targets. In this strategy, one aptamer is immobilized on the surface of a sensor, while a secondary labeled aptamer is used for signal amplification. The target is sandwiched between them. A target must have two independent binding sites for aptamers that may bind to it without affecting the binding of other targets. In the "signal-off" aptasensor format, the detectable signals decrease as the target concentration increases. In this case, a strong background signal toward zero target concentration is necessary. In contrast, sandwich-format aptasensors display a "signal-on" mode with the target concentration increased. Very few targets have two independent binding sites and two

or more isolated aptamers, which limits the application of this type of sensor format. However, this limitation can be resolved by the use of two formats, namely aptamer-protein-aptamer (involving the same aptamer) and aptamer-protein-antibody [31,95]. In 2016, Mazloum-Ardakani and Hosseinzadeh [96] designed an enzyme-free sandwich-type electrochemical aptasensor for the sensitive detection of tumor necrosis factor-alpha (TNF-α) (Fig. 6). In this work, bimetallic silver@platinum core-shell nanoparticles-functionalized graphene nanosheets were applied as labels for signal amplification, and gold nanoparticles-functionalized graphene nanosheets/chitosan nanocomposite (Au-GRs/CS) served as a sensing platform. The direct immobilization of a thiolated aptamer took place at a 5′-terminal on a self-assembled monolayer on an Au-GRs/CS-modified screen-printed working electrode through a platinum-sulfur interaction. For the detection of TNF-α, the aptasensor was incubated with a varying concentration of TNF-α, and then the Aptamer-Ag@Pt-GRs bioconjugate was placed on the modified electrode to form a sandwich-format aptasensor. The electrochemical measurements of this aptasensor for TNF-α samples were carried out through catechol as a probe. Since the Ag@Pt-GRs label has good electrocatalytic activity for the oxidation of catechol, the proposed sandwich-format "signal-on" aptasensor can successfully be used to detect TNF-α in human serum samples. In 2014, Liu et al. [97] constructed a sandwich electrochemiluminescence (ECL) aptasensor for the sensitive detection of thrombin based on molybdenum disulfide nanosheet–graphene (MoS2–GR)

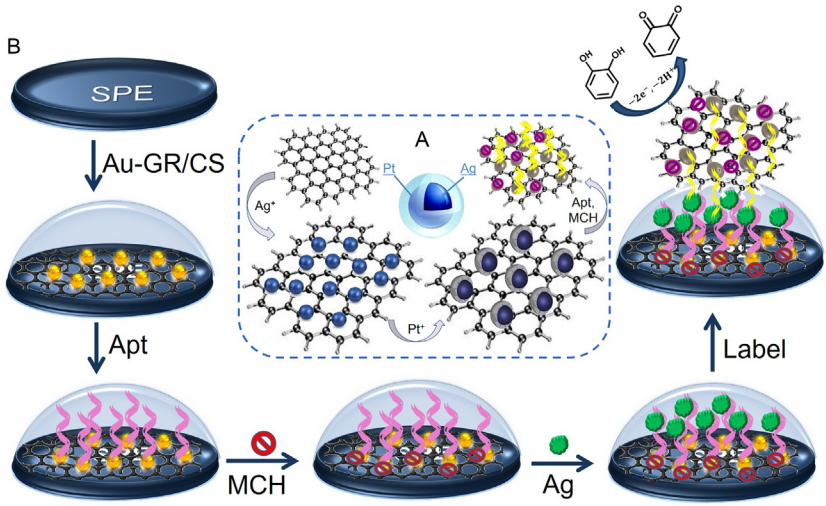

Fig. 6 (A) Preparation of label. (B) The schematic diagram for the preparation of TNF-α aptasensor.

composites and gold nanoparticles. To fabricate the aptasensor, a glassy carbon electrode was modified by MoS2–GR nanocomposite and put in a 3-aminopropyltriethoxysilane (APTS) solution to introduce amine functional groups. Then, the electrode was covered with gold nanoparticles to immobilize a thiol-modified first aptamer through a gold-sulfur bond interaction on the modified electrode surface. The carboxylic groups on CdSe–ZnS quantum dots were activated with EDC and NHS, and an amine-terminated second aptamer was covalently coupled to the carboxylic acid groups of the quantum dots. For the detection of thrombin, the first aptasensor was incubated with a varying concentration of thrombin, and then the second aptamer labeled with the quantum dots was placed on the modified electrode to form a sandwich-format aptasensor. This sandwich-format "signal-on" aptasensor was successfully applied to the determination of thrombin in human plasma. In 2019, Bai et al. [98] reported a sandwich-type electrochemical aptasensor for the rapid and sensitive determination of *Mycobacterium tuberculosis* MPT64 antigen (Fig. 7). In this work, a novel nanocomposite consisting of fullerene nanoparticles, nitrogen-doped carbon nanotubes and graphene oxide (C60NPs-N-CNTs/GO) was synthesized. The surface of the nanocomposite was covered with gold nanoparticles through a gold-nitrogen bond interaction, and it was coupled to a thioled second aptamer through a gold-sulfur bond interaction. In the same way, a thioled second aptamer was labeled with Au/C60NPs-N-CNTs/GO. Then, a conductive polyethyleneimine (PEI)-functionalized Fe-based metal-organic framework (P-MOF) was applied as a sensing platform to absorb

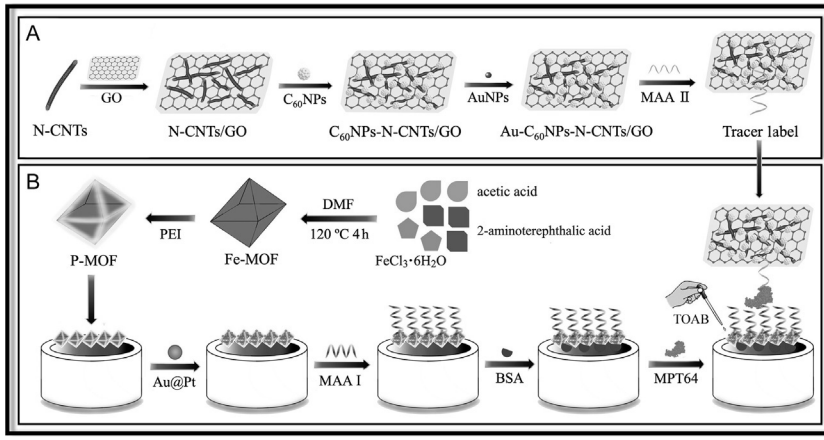

Fig. 7 (A) Illustration of the preparation procedure of the tracer label. (B) Schematic representation of the self-assembly process of the proposed aptasensor.

bimetallic core-shell gold-platinum nanoparticles (Au@Pt). The thiolated first aptamer was directly immobilized on the Au@Pt nanoparticles through a platinum-sulfur interaction. To construct a typical sandwich-type aptasensor, the thiolated first aptamer was immobilized on a modified glass carbon electrode. Thereafter, the electrode was immersed in varying concentrations of an antigen and then in the second labeled aptamer solution. The electrochemical measurements of this aptasensor for the antigen samples were carried out through tetraoctylammonium bromide as a probe. This is because the tracer label has good electrocatalytic activity for tetraoctylammonium bromide.

4.2 Label-free aptasensors

In this category, the biological recognition element is not labeled; instead, the aptamers are immobilized on the sensor surface and the target-aptamer interaction is detected by different techniques [99–103]. A variety of techniques for target measurement have been used in the label-free strategy.

In 2015, Meirinho et al. [104] reported a simple and label-free voltammetric aptasensor for the detection of osteopontin (OPN). A biotinylated RNA aptamer was immobilized on a streptavidin-modified screen-printed gold electrode through a streptavidin-biotin interaction. The electrochemical behavior of the electrode surface after each preparation step of the aptasensor was studied through CV and square wave voltammetry. In this case, the Ferro/ferricyanide solution was as a redox probe. After the immobilization of aptamers and the incubation of protein solutions, the current decrease was measured by an electrochemical technique. This "signal-off" label-free aptasensor had sufficient sensitivity and selectivity for the detection of OPN in the presence of other interfering proteins, except for thrombin. In 2019, Zhou et al. [105] fabricated a label-free fluorescence aptasensor for the good selective determination of cadmium. In this work, SYBR green I (SG), as a probe, could bind to the small groove of double-stranded DNA. This involved an aptamer and a complementary sequence and generated high fluorescence intensity. In the absence of cadmium ions, double-stranded a DNA-SG complex generated high fluorescence intensity. After addition of cadmium ions, the special high-affinity binding of cadmium to the aptamer denatured the double-stranded DNA, and the conformational switching of the aptamer from dsDNA to a stem-loop structure changed the fluorescence intensity. This "signal-on" label-free aptasensor, which was based on the conformational changing of the aptamer, proved to have good selectivity and

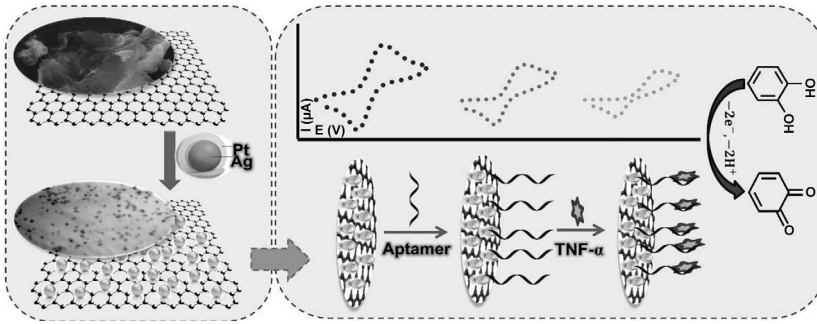

Fig. 8 The schematic diagram for the preparation of TNF-α aptasensor.

sensitivity for the detection of cadmium in real samples. In 2015, Mazloum-Ardakani et al. [106] reported a sensitive and simple label-free electrochemical aptasensor for the detection of tumor necrosis factor-alpha (TNF-α) (Fig. 8). They synthesized bimetallic silver/platinum core-shell nanoparticles supported on reduced graphene oxide nanosheets (Ag@Pt-GRs) as a novel desirable sensor platform. The sensor was used as an electrocatalyst for catechol, which was a probe in this aptasensor. In this work, gold screen-printed electrodes were modified with Ag@Pt-GRs and then with a thiolated aptamer at the 5′-terminal. These materials were immobilized on the modified electrode surface through a platinum-sulfur interaction. For the detection of TNF-α, the aptasensor was incubated with a varying concentration of TNF-α, and the electrochemical measurements of the aptasensor were carried out in a catechol solution as a probe. With an increase in the TNF-α concentration, the electrochemical signal decreased because the electrode surface was blocked with the biological elements. This "signal-off" label-free aptasensor was based on the electrocatalytic effect of nanocomposite on the electrode surface. It was successfully used to detect TNF-α in human serum samples. In 2019, Wen et al. [107] fabricated a novel label-free electrochemical impedance aptasensor for the highly sensitive detection of interferon-gamma (IFN-γ) based on target-induced exonuclease inhibition. The thiolated aptamer was immobilized on the surface of a gold electrode at the 5′-terminal through a gold-sulfur interaction. Then, the aptasensor was placed in a solution consisting of exonuclease III (Exo III) and exonuclease I (Exo I). In the absence of IFN-γ, Exo III and Exo I decomposed the double-stranded and single-stranded sequences of the aptamer, respectively. Also, smaller electron-transfer resistance (Rct) was observed on the electrode surface. In the presence of IFN-γ, the function of Exo III was inhibited after the three bases of the aptamer were cut due to the binding of the aptamer with the IFN-γ. As different concentrations of IFN-γ were added, the Rct was further increased. This label-free

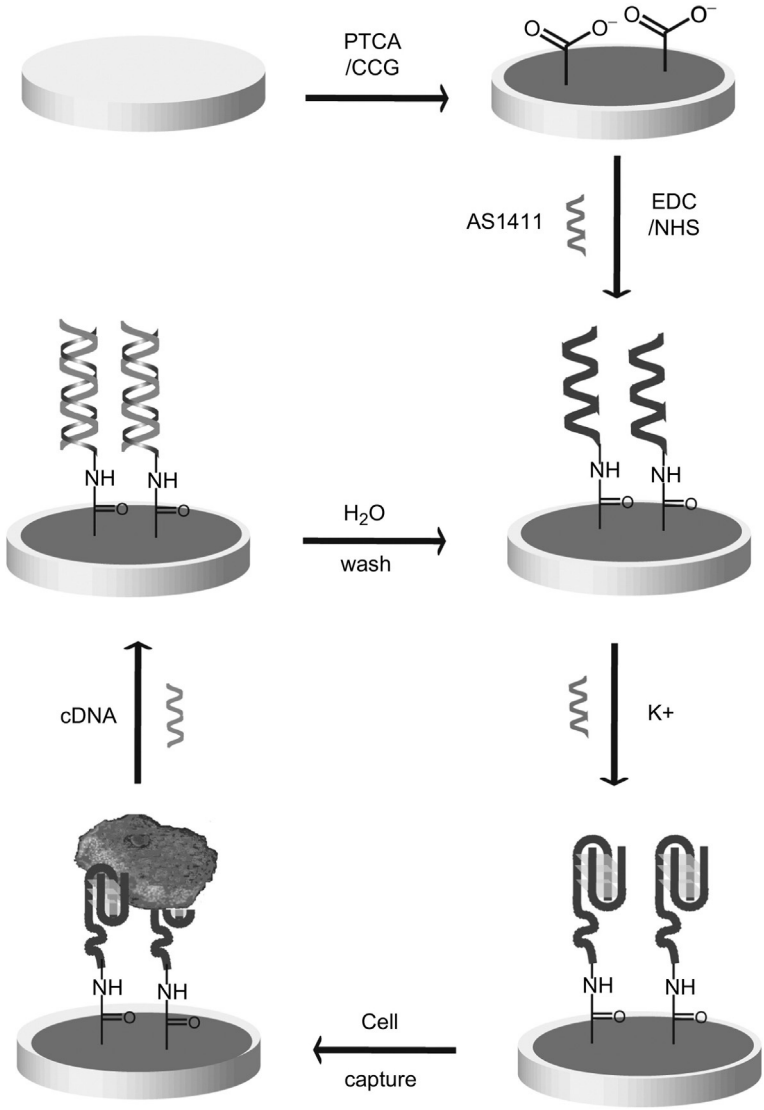

Fig. 9 Schematic representation of the reusable aptamer/graphene-based aptasensor.

"signal-on" aptasensor was able to detect IFN-γ in serum samples successfully. Electrochemical impedance spectroscopy was conducted to fabricate label-free biosensors by monitoring the increase in the electron-transfer resistance (Rct) of the electrode [108].

In 2011, Qu et al. [109] reported an electrochemical aptasensor for cancer cell detection (Fig. 9). In this work, a glassy carbon electrode was modified

with a nanocomposite that consisted of hydrolyzing 3,4,9,10-perylene tetracarboxylic dianhydride, graphene, and Nafion (PTCA/CCG-Nafion). Amine-terminated aptamers were covalently coupled to the carboxylic acid groups of the PTCA/CCG composite on the electrode surface and subsequently hybridized with aptamer DNA. The resulting aptasensor was used to capture cells on the electrode surface by the specific bond between the cell surface nucleolin and the aptamer. When hybridized with the aptamer complementary DNA, the aptamer adopted a different conformation, and it could not bind to the overexpressed nucleolin on the plasma membrane of cancer cells. After a mild wash, the electrode became reusable for detection. In other words, the DNA hybridization process could regenerate the aptasensor for cancer cell detection. Electrochemical impedance spectroscopy was done in the fabricating process of this aptasensor by the monitoring of the electron-transfer resistance (Rct) of the electrode with an increase in the cancer cell concentration.

5. Target analytes

In this section of the study, a few examples are presented of the aptasensors applied in clinical diagnostics or those that have potential applications in clinical diagnostics.

5.1 Biomarkers

Biomarkers are biological molecules found in human fluids such as blood, urine and other tissues. They are signs of normal or abnormal processes and of certain conditions or diseases. Biomarkers, i.e., the informative signals derived from the body, are used in clinical practice for the diagnosis of diseases and the prognosis of how the body responds to the treatment of those diseases. They can, indeed, serve as a guide for treatment. Thus, the quantification of biomarkers in the human body is of great importance for clinical diagnosis and biomedical research [110–112]. In 2016, Heli et al. [113] fabricated a label-free electrochemical aptasensor for the detection of prostate-specific antigen (PSA) in the blood serum samples of healthy and sick persons. In this work, gold nanospheres were electrodeposited on a gold electrode, and thiolated aptamers were self-assembled through dithiothreitol on the modified electrode surface. For the detection of PSA, the aptasensor was incubated with a varying concentration of PSA, and then it was incubated in a solution of methylene blue (MB). Electrochemical detection was performed by CV and DPV for the reduction peak of the bonded MB.

In 2019, Villalonga et al. [114] designed an amperometric aptasensor for PSA detection using a functionalized graphene-modified carbon screen-printed electrode as a platform. The carbon screen-printed electrode was coated with carboxylic-functionalized graphene. The carboxylic groups on graphene were activated with EDC and NHS, and amine-terminated anti-PSA aptamers were covalently coupled to the carboxylic acid groups of graphene. Thereafter, partially complementary DNA (cDNA) was hybridized with the anti-PSA aptamer through the intercalation of MB into the double-stranded DNA sequences. This could be done due to its affinity to guanine. After the target was added, the special high-affinity binding of PSA to the aptamer lead to the denaturation of the double-stranded DNA and a decrease in the signal of MB that was intercalated between the two sequences. The quantification of PSA was measured using DPV.

Aptasensors have been used for the detection of various biomarkers. Some of the reported aptasensors for the biomarker detection mention in Table 1.

5.2 Cancer cells

Factors that enable cancer cells to move and grow outside a primary site and those that control the timing of tumor cell dispersion are still not well understood. Tumor metastasis involves a series of steps in which cells are detached from primary tumors and move to distant sites through physiological fluids. These cells are named circulating tumor cells (CTCs). Many studies have been conducted on disseminated tumor cells (DTCs) in bone marrow and CTCs in blood. Since the treatment of bone marrow is time-consuming and uncomfortable for patients, researchers have recently focused on the detection of CTCs in the blood of cancer patients [145–148].

SELEX process can be employed to produce aptamers for target CTCs. It is assumed that one aptamer binds to one specific antigen on the cell surface, and several different aptamers can recognize the cell phenotype with high binding affinity. This allows aptamers to distinguish cell type, malignant status, cancer type, proliferation capability, and metastatic potential. Furthermore, owing to their high specificity for targets, low toxicity and many advantages over other biological recognition elements, aptamers can be considered appropriate for cancer cell recognition [149–151].

In 2013, Song et al. [152] reported an electrochemical aptasensor for the detection of human breast cancer cells. In this work, a glassy carbon electrode was modified by synthesized porous graphene oxide/gold nanocomposites

Table 1 Application of aptasensors for detection of biomarkers.

Biomarker	Strategy type	Signal transduction	Linear range	LOD	Reference
MUC1	Label-free aptasensor	Electrochemical	8.8–353.3 nM	2.2 nM	[115]
MUC1	Label-free aptasensor	Electrochemical	0.1 pM–10 nM	0.033 pM	[116]
MUC1	Label-free aptasensor	EIS	0.5–10 nM	0.1 nM	[117]
MUC1	Label aptasensor	Fluorescent	0.04–10 μM	28 nM	[118]
PSA	Label-free aptasensor	EIS	1–10 ng/mL	–	[119]
PSA	Label aptasensor	SERS	5–500 pg/mL	5.0 pg/mL	[120]
PSA	Label-free aptasensor	Electrochemical	1–300 ng/mL	280 pg/mL	[121]
PSA	Label-free aptasensor	Fluorescent	0–200 ng/mL	0.5 ng/mL	[122]
Thrombin	Label-free aptasensor	EIS	1–50 nM	0.01 nM	[123]
Thrombin	Label aptasensor	Electrogenerated chemiluminescence	10 fM–10 pM	1.0 fM	[124]
Thrombin	Label aptasensor	Electrochemical	1 pM–10 nM	0.64 pM	[29]
Thrombin	Label-free aptasensor	Electrochemical	5 pM–5 nM	1.8 pM	[125]
IFN-γ	Label-free aptasensor	Electrochemical	0.1–0.7 pM	0.065 pM	[126]
IFN-γ	Label-free aptasensor	SPR	0.01–100 nM	10 pM	[127]
IFN-γ	Label-free aptasensor	EIS	1–5 ng/mL	11.56 pM	[128]

IFN-γ	Label aptasensor	Fluorescent	3.5 fM–1.0 pM	1.5 fM	[129]
HER2	Label-free aptasensor	Electrochemical	0.1–500 pg/mL	0.05 pg/mL	[130]
HER2	Label-free aptasensor	EIS	10^{-5}–10^{2} ng/mL	–	[131]
HER2	Label-free aptasensor	Electrochemical	10–60 ng/mL	3.0 ng/mL	[132]
HER2	Label aptasensor	Electrochemical	10–150 ng/mL	4.9 ng/mL	[133]
IgE	Label-free aptasensor	EIS	0.03–42.8 μg/mL	0.03 μg/mL	[134]
IgE	Label-free aptasensor	Electrochemical	0.5–30 nM	37 pM	[135]
IgE	Label-free aptasensor	Electrochemical	1–100,000 pM	0.16 pM	[136]
IgE	Label-free aptasensor	Colorimetry	1–25 nM	0.2 nM	[137]
TNF-α	Label aptasensor	Electrochemical	Extending to 6 nM	58 pM	[138]
TNF-α	Label-free aptasensor	Electrochemical	0.001–100 ng/mL	0.5 pg/mL	[139]
TNF-α	Label-free aptasensor	Electrochemical	10 pg/mL–40 μg/mL	5.5 pg/mL	[140]
TNF-α	Label aptasensor	Electrochemical	1–100 pg/mL	0.52 pg/mL	[141]
CEA	Label-free aptasensor	FRET	0.1–40 ng/mL	–	[142]
CEA	Label aptasensor	Electrochemical	0.1 pg/mL–10 ng/mL	0.05 pg/mL	[143]
CEA	Label-free aptasensor	Electrochemical	0.0001–10 ng/mL	40 fg/mL	[143]
CEA	Label aptasensor	Electrochemical	0.5 fg/mL–0.5 ng/mL	0.34 fg/mL	[144]

(GO/Au nanocomposites). 3′-amine-terminated MUC1-binding aptamers were immobilized on the modified electrode surface based on the interaction between the NH_2 groups on the aptamer and gold. Thionine/PtFe composites were applied as labels for signal amplification. For the detection of cancer cells, the aptasensor was incubated with a varying concentration of cells, and then the aptamer-thionine/PtFe bioconjugate was placed on the modified electrode to form a sandwich-format aptasensor. The electrochemical measurements of this aptasensor were carried out for cancer cell samples through the electrochemical signals of thionine. This sandwich-format "signal-on" aptasensor was successfully used to detect cancer cells samples. In 2016, Chen et al. [153] demonstrated an electrochemical aptasensor for the detection of breast cancer cells via a free-running DNA walker. In this assay, short thiolated D-RNA was immobilized on a gold electrode through a gold-sulfur interaction. Then, the aptamer, DNA walker and streptavidin magnetic beads (SA-Mbs) were mixed at room temperature to obtain DNA walker/Ap/SA-Mbs. Afterwards, a cancer cells solution was added to that resultant. Finally, the complex was separated with a magnet, and the DNA walker supernatant was gained. In another attempt, DNA walker was added onto the modified electrode and hybridized by D-RNA on the electrode surface. In the presence of cancer cells, the DNA walker was denatured from the double-strand DNA walker/aptamer because of high-affinity binding between the cancer cell and the aptamer. The released DNA walker was cast on the D-RNA/gold electrode to form the double-strand DNA walker/D-RNA with a catalytic core. Then, the DNA walker cut the cleavage point rArU of the D-RNA sequence in the presence of magnesium ions, releasing a short D-RNA fragment and leaving the DNA walker with a free recognition arm. Afterward, the DNA walker was preferably hybridized with another adjacent D-RNA through a sequence displacement interaction. It triggered the formation of more energy-favorable double-strand structures and another round of catalytic cleavage. Finally, considerable signal amplification was achieved. In the absence of cancer cells, the above process was inhibited, and there was no change in the signal. In 2016, Hosseini et al. [154] designed a simple and sensitive colorimetric aptasensor for cancer cells detection. First, two thiolated probe sequences were bound to gold nanoparticles through a gold-sulfur bond interaction. Then, a solution containing two types of functionalized nanoparticle probes was added to the aptamer solution, and hybridization was done. For the detection of cancer cells, the cells were added to the mentioned solution resultant, and the aptamer was incubated with cancer cells. In this way, the aptamer released probe/gold nanoparticle dimers,

Table 2 Application of aptasensors for detection of cancer cells.

Cancer cells	Strategy type	Signal transduction	Linear range	LOD	Reference
Breast cancer	Label free	Electrochemical	50–10^6 cells/mL	20 cells/mL	[155]
Leukemia	Label free	Fluorescent	5–5×10^6 cells/mL	5 cells/mL	[156]
CCRF-CEM	Label free	Colorimetric	10^2–10^4 cells/mL	40 cells/mL	[157]
CCRF-CEM	Label aptasensor	Fluorescence	50–10^5 cells/mL	25 cells/mL	[158]
K562 cancer	Label aptasensor	Electrochemical	14–1.4×10^6 cells/mL	14 cells/mL	[159]
Breast cancer	Label free	Photoelectrochemical	10^3–10^5 cells/mL	400 cells/mL	[160]
Ramos cancer	Label free	ECL	100–1000 cells/mL	58 cells/mL	[161]

and the separated gold nanoparticles appeared in a red color. With an increase in the cancer cells concentration, more aptamer was removed and more separated gold nanoparticles appeared in the solution. Thus, the cells were detected by a colorimetric aptasensor.

Some of the aptasensors reported for the detection of cancer cells are mentioned in Table 2.

5.3 Microorganisms and viruses

Viruses are infectious agents with a simple composition and a small size that multiply only inside the living cells of other organisms such as animals, plants and microorganisms, including bacteria. The early diagnosis of infectious agents such as viruses is of great importance for clinical diagnosis because viruses have a very small size and can rapidly infect all living beings (i.e., humans, animals, and plants). To date, many efforts have been taken to design rapid and accurate monitoring approaches for the detection of virus-related targets [162,163]. In this regard, the use of aptamers for virus recognition has been favored. This is owing to their advantages over other biological recognition elements. Among the advantages of aptasensors, one may refer to chemical stability over a wide range of pH, storage conditions and temperature, fast generation, high specificity for targets, and low toxicity [164–166]. In 2012, Berezovski et al. [167] fabricated a label-free aptasensor for live vaccinia virus (VACV) detection. In this work, thiolated complementary single-stranded DNA was self-assembled on the surface of a

gold electrode through a gold-sulfur interaction. Then, a VACV-binding aptamer was hybridized with the complementary single-stranded DNA and immobilized on the electrode surface. This aptasensor proved to be highly selective and could distinguish viable vaccinia virus particles from nonviable viruses. EIS was conducted to detect the binding event between the VACV-binding aptamer and the target VACV. The proposed label-free electrochemical aptasensor was successfully used to detect VACV in human blood plasma. In 2017, Li et al. [40] presented a label-free QCM aptasensor for the detection of H5N1 avian influenza virus (AIV). First, a prepared nanoporous gold film was self-assembled on the surface of a gold electrode by 1,6-hexanedithiol as a bifunctional cross-linker. A monolayer of 16-mercaptohexadecanoic acid (MHDA) was attached to the gold film through a gold-sulfur interaction. The carboxylic groups of MHDA were activated with EDC and NHS, and amine-terminated AIV-binding aptamers were covalently coupled to the carboxylic acid groups of MHDA. The amine-terminated AIV-binding aptamers were, thus, immobilized through the amide bond on the electrode surface. For the detection of AIV, the aptasensor was incubated with a varying concentration of AIV, and the change of the resonant frequency was detected. The developed assay could be applied for the detection of other viruses as well. In 2016, Li et al. [168] reported an impedance aptasensor for the detection of H5N1 avian influenza virus. In this study, a biotinylated H5N1-binding aptamer was immobilized on the surface of a streptavidin-coated interdigitated array (IDA) microelectrode via specific noncovalent interactions and by using biotin-avidin. The aptasensor captured the virus, and the variation of impedance due to the captured virus was measured using an impedance analyzer. To amplify the signal, a gold nanoparticles/protein cross-linker/aptamer/thiocyanic acid composite was fabricated and added to bind the viruses on the electrode surface. The developed aptasensor could be applied for the detection of other viruses too.

Some of the aptasensors reported for the detection of viruses are mentioned in Table 3.

5.4 Drugs

As generally defined, a drug is any substance used as a medicine or in the preparation of a medicine that has a physiological effect when it enters the body. Because of the effects of drugs on the body during treatment and after that, it is of great importance to detect and trace them in a simple, rapid, cost-effective way and with a small amount of biological sample. In addition, drug sensors are needed especially for drugs with many side effects

Table 3 Application of aptasensors for detection of viruses.

Viruses	Strategy type	Signal transduction	Linear range	LOD	Reference
Avian influenza virus	Label free	SPR	0.128–1.28 HAU	0.128 HAU	[169]
Hepatitis C virus	Label free	EIS	10–70 pg/mL	3.3 pg/mL	[170]
Influenza virus	Label aptasensor	SERS	–	10^{-4} HAU	[171]
HTLV-II	Label free	EIS	1 pM–1 nM	0.171 pM	[172]
Vibrio parahaemolyticus	Label aptasensor	Fluorescent	$14–1.4 \times 10^6$ cfu/mL	7 cfu/mL	[173]

such as anti-cancer drugs. In this respect, aptasensors can play an important role to determine drugs in biological samples. The capability of these sensors is due to the unique advantages of aptamers such as ease of synthesis, commercial availability, high specificity, affinity to various target molecules, chemical stability in experimental conditions, and ease of chemical modification with different reporter tags [174–176]. In 2014, Liu et al. [177] designed a label-free aptasensor for the electrochemical detection of tetracycline. Tetracycline (TET) is an antibiotic that fights infections caused by bacteria. The TET-binding aptamer was immobilized on the surface of a gold electrode through an interaction between gold and the NH_2 groups on the aptamer. Before the measurements, the relationship between TET concentration and electron-transfer resistance (Ret) was analyzed by EIS. After the addition of various concentrations of TET, the Ret was found increased. This label-free "signal-on" electrochemical aptasensor was able to detect TET in milk samples successfully. In 2014, Zhao et al. [178] developed an electrochemical impedance aptasensor for the detection of 17β-estradiol (E2). 17β-estradiol is a steroidal human hormone not only involved in sexual maturation and reproduction but also playing a myriad of important roles throughout the body. In this study, at first, zinc nanoparticles were electrodeposited on a boron-doped diamond (BDD) electrode. Then, Zn nanoparticles were applied as the second template for the growth of dendritic gold. A dendritic gold nanostructure was, thus, obtained on a BDD substrate (i.e., a dendritic gold/BDD electrode). The direct immobilization of a thiolated E2 aptamer led to the formation of a self-assembled monolayer on the dendritic gold/BDD electrode through a gold-sulfur interaction. With the increase of the E2

concentration, the Ret was increased too due to the formation of more analogous insulating layers. Thus, E2 could be detected by the EIS technique. This electrochemical aptasensor was successfully applied to detect E2 in real water samples. In 2017, Emrani et al. [179] constructed an electrochemical aptasensor for the ultrasensitive detection of fluoroquinolones. Fluoroquinolones are antibiotics widely used to treat respiratory and urinary tract infections. First, a thiolated aptamer was immobilized on a gold electrode through a gold-sulfur interaction. For electrochemical measurements, different concentrations of the drug were incubated on the surface of the aptasensor, and then the single-stranded DNA-binding protein (SSB) was added to the apt-modified electrode surface. In the absence of the target, SSB could bind to the aptamer and a weak electrochemical signal could be observed due to the steric hindrance and electrostatic repulsive force. However, the electrochemical signal increased as the concentration of the incubated target rose on the aptasensor. This occurred due to the better access of the probe to the surface of the electrode. The proposed electrochemical aptasensor was successfully used to detect fluoroquinolones in serum, milk and water samples.

Some of the aptasensors reported for drug detection are mentioned in Table 4.

5.5 Other targets

In addition to the above mentioned four types of targets as the most important ones, several other compounds (such as metal ions, toxins, and pesticides) have been studied by means of aptasensors. Some of the aptasensors reported for the detection of these targets in recent years are mentioned in the following.

Table 4 Application of aptasensors for detection of drugs.

Drugs	Strategy type	Signal transduction	Linear range	LOD	Reference
Ampicillin and kanamycin A	Label free	EIS	100 pM–1 μM and 10 nM–1 mM	–	[180]
Diclofenac	Label free	EIS	10–200 nM	2.7 nM	[181]
Ampicillin	Label free	EC-SPR	2.5–1000 μM	1 μM	[47]
Sulfamethazine	Label free	Chemiluminescence	1.85–21.57 ng/mL	0.92 ng/mL	[182]
Sulfadimethoxine	Label free	Fluorescence	10–250 ng/mL	1.54 ng/mL	[183]
Tetracycline	Label free	Electrochemical	0.05–20 μg/L	0.035 μg/L	[176]

In 2019, Bai et al. [184] reported a label-free aptasensor for the detection of cadmium ions. The aptasensor was developed based on the immobilization of a thiolated aptamer on a gold screen-printed electrode through a gold-sulfur interaction. This electrochemical aptasensor was used for cadmium detection by dropping a cadmium solution on the screen-printed electrode. The CV and DPV signal values decreased after the incubation of the cadmium ions on the aptasensor surface. It was due to the increase in the electron-transfer resistance of the electrode interface. Finally, this label-free electrochemical aptasensor was successfully used to detect cadmium ions. In 2019, Wei et al. [185] developed a sensitive electrochemical aptasensor for the detection of lead ions (Pb^{2+}). In this study, a glass carbon electrode was firstly modified with gold-modified porous reduced graphene oxide. Then, a thiolated aptamer was immobilized on the modified electrode, and the resulting aptasensor was applied to lead detection in a sandwich assay. Another thiolated aptamer was labeled with gold-modified graphene oxide (Au NPs@GO). To detect lead ions, the aptasensor was incubated with a varying concentration of lead ions, and then aptamer-Au NPs@GO bioconjugate was placed on the modified electrode to form a sandwich-format aptasensor. The electrochemical measurements of this aptasensor were carried out for the lead ion samples through H_2O_2 as a probe. H_2O_2 was used in this case because Au NPs@GO, as a signal probe, exhibits high electrocatalytic activity for H_2O_2. This sandwich-format aptasensor was successfully used to detect lead ions. In 2019, Wang et al. [186] designed a label-free electrochemical aptasensor for the detection of malachite green (MG). MG is a triphenylmethane dye widely applied in aquaculture. At first, a glassy carbon electrode was modified with a gold nanoparticles/graphene quantum dots-tungsten disulfide nanosheets composite film and used as a sensing platform for the immobilization of thiolated MG-binding aptamers. After MG was incubated on the aptasensor, due to the affinity of the aptamer to MG, a well-defined anodic peak resulted from the oxidation of MG on the modified electrode. The peak current related to the MG oxidation was used to determine MG through DPV. In 2019, Yongqiang et al. [187] fabricated an electrochemical aptasensor for the detection of acetamiprid. Acetamiprid is an insecticide in the class of neonicotinoids that causes paralysis and death of pests via activating nicotinic receptors. It is highly toxic to birds and earthworms and moderately toxic for most aquatic organisms and mammals. In this study, a mixture of graphene aerogel (GA) and a chitosan solution was dropped on the surface of a glassy carbon electrode. Then, the Glu-GQD/Au dispersion in chitosan was added onto the modified electrode surface. This platform was used to immobilize a thiolated

acetamiprid-binding aptamer through a gold-sulfur interaction on the modified electrode surface. The Glu-GQD/Au served as a probe to produce sensitive electrochemical responses to acetamiprid. After the incubation of different concentrations of acetamiprid on the aptasensor surface, the peak currents in both CV and DPV curves decreased, making the sensor applicable for acetamiprid detection. This aptasensor was successfully used to detect acetamiprid in vegetables. In 2019, Prabhakar et al. [188] developed an electrochemical aptasensor for the detection of ochratoxin A (OTA). Ochratoxin A (OTA) is a toxic secondary metabolite produced mainly by filamentous fungus species in various kinds of foodstuffs. In this work, an electrode was prepared by the dip-coating of ITO sheets in a chitosan-graphene suspension. Then, the electrode was modified with streptavidin through the binding of the activated carboxylic groups of graphene to the NH_2 groups of streptavidin. A biotinylated aptamer was immobilized on the surface of the modified electrode through a biotin-streptavidin interaction. To detect OTA, the aptasensor was incubated with a varying concentration of OTA. Through DPV, a decrease was observed in the peak current, which was due to the blocking of the electrode surface with the biological elements. This electrochemical aptasensor was successfully used to detect OTA in grape juice samples.

With the advantages of aptamers, a wide range of aptasensors can be developed for the analysis of different targets. The major challenge is to achieve aptamers with high affinity and specificity for new targets. Based on recent studies, aptasensors can be appropriate platforms for the detection of various targets. It is possible to envision the successful use of aptasensors for clinical and medical analyses in the future (Table 5).

Table 5 Application of aptasensors for other targets.

Targets	Strategy type	Signal transduction	Linear range	LOD	Reference
As^{3+}	Label free	Fluorescence	10 pM–1 μM	2 pM	[189]
Hg^{2+}	Label aptasensor	Electrochemical	0.1–100 nM	30 pM	[190]
Anatoxin-a	Label free	EIS	1–100 nM	0.5 nM	[191]
Ochratoxin A	Label free	EIS	0.01–100 nM	14 pM	[192]
Chlorpyrifos	Label free	Electrochemical	1–10^5 ng/mL	0.33 ng/mL	[193]
Malathion	Label free	Electrochemical	–	0.001 ng/L	[194]

6. Summary and future perspectives

This chapter presents an overview of the aptasensors recently developed for the analysis of a wide range of target analytes such as biomarkers, cancer cells, microorganisms, viruses, and drugs. The discovery of aptamers was a major step toward new horizons not only in biosensing but also in clinical diagnostics. The advantages of aptamers such as easy chemical modification, flexibility, and high affinity provide the opportunity to immobilize them on the surface of electrodes through various methods and to adopt different strategies to design appropriate biosensors for the detection of a wide range of targets. Most of the biosensors reported in this study have exhibited remarkable features, such as high sensitivity and selectivity as well as potential applicability in clinical diagnostics. By taking the advantage of new techniques, it is possible to improve aptasensors and to integrate them into portable devices. Recently, aptasensors have been developed as a good alternative for immunosensors, which is due to the preference of aptamers over antibody especially in terms of the cost of production and the degree of robusticity. In designing aptasensors, however, there are still some major challenges to overcome. These challenges will, indeed, make the future of biosensing. One may refer to some cases in this regard. First, certain aptasensors should be developed with the capability of real-time monitoring to understand how pathogens invade and destroy the human body. Second, since the development of novel aptasensors depends on the synthesis of aptamers for new target analytes with high affinity and specificity, devising new methods of aptamer selection can considerably improve the field of aptasensor fabrication. Finally, it is desirable to develop aptasensors with the capability of detecting more than one target simultaneously.

References

[1] M. Mazloum-Ardakani, L. Hosseinzadeh, A. Khoshroo, Label-free electrochemical immunosensor for detection of tumor necrosis factor α based on fullerene-functionalized carbon nanotubes/ionic liquid, J. Electroanal. Chem. 757 (2015) 58–64.

[2] S. Galic, J.S. Oakhill, G.R. Steinberg, Adipose tissue as an endocrine organ, Mol. Cell. Endocrinol. 316 (2010) 129.

[3] S. Dehghani, R. Nosrati, M. Yousefi, A. Nezami, F. Soltani, S.M. Taghdisi, K. Abnous, M. Alibolandi, M. Ramezani, Aptamer-based biosensors and nanosensors for the detection of vascular endothelial growth factor (VEGF): a review, Biosens. Bioelectron. 110 (2018) 23–37.

[4] T. Šmuc, I.-Y. Ahn, H. Ulrich, Nucleic acid aptamers as high affinity ligands in biotechnology and biosensorics, J. Pharm. Biomed. Anal. 81–82 (2013) 210–217, https://doi.org/10.1016/j.jpba.2013.03.014.

[5] S. Tombelli, M. Minunni, M. Mascini, Analytical applications of aptamers, Biosens. Bioelectron. 20 (2005) 2424–2434, https://doi.org/10.1016/j.bios.2004.11.006.
[6] Y.C. Lim, A.Z. Kouzani, W. Duan, Aptasensors: a review, J. Biomed. Nanotechnol. 6 (2010) 93–105.
[7] A. Mokhtarzadeh, J.E.N. Dolatabadi, K. Abnous, M. de la Guardia, M. Ramezani, Nanomaterial-based cocaine aptasensors, Biosens. Bioelectron. 68 (2015) 95–106. https://doi.org/10.1016/j.bios.2014.12.052.
[8] C. Feng, S. Dai, L. Wang, Optical aptasensors for quantitative detection of small biomolecules: a review, Biosens. Bioelectron. 59 (2014) 64–74.
[9] B. Kudłak, M. Wieczerzak, Aptamer based tools for environmental and therapeutic monitoring: a review of developments, applications, future perspectives, Crit. Rev. Environ. Sci. Technol. 50 (2019) 1–52.
[10] M. Zayats, Y. Huang, R. Gill, C. Ma, I. Willner, Label-free and reagentless aptamer-based sensors for small molecules, J. Am. Chem. Soc. 128 (2006) 13666–13667.
[11] J.H. An, S.J. Park, O.S. Kwon, J. Bae, J. Jang, High-performance flexible graphene aptasensor for mercury detection in mussels, ACS Nano 7 (2013) 10563–10571.
[12] P. Tong, W.-W. Zhao, L. Zhang, J.-J. Xu, H.-Y. Chen, Double-probe signal enhancing strategy for toxin aptasensing based on rolling circle amplification, Biosens. Bioelectron. 33 (2012) 146–151.
[13] H. Qin, J. Liu, C. Chen, J. Wang, E. Wang, An electrochemical aptasensor for chiral peptide detection using layer-by-layer assembly of polyelectrolyte-methylene blue/polyelectrolyte-graphene multilayer, Anal. Chim. Acta 712 (2012) 127–131.
[14] C. Ibau, M.K.M. Arshad, S.C.B. Gopinath, M. Nuzaihan, M.F.M. Fathil, P. Estrela, Gold interdigitated triple-microelectrodes for label-free prognosticative aptasensing of prostate cancer biomarker in serum, Biosens. Bioelectron. 136 (2019) 118–127.
[15] C.D. Medley, S. Bamrungsap, W. Tan, J.E. Smith, Aptamer-conjugated nanoparticles for cancer cell detection, Anal. Chem. 83 (2011) 727–734.
[16] A.D. Ellington, J.W. Szostak, In vitro selection of RNA molecules that bind specific ligands, Nature 346 (1990) 818–822.
[17] F. Li, Z. Yu, X. Han, R.Y. Lai, Electrochemical aptamer-based sensors for food and water analysis: a review, Anal. Chim. Acta 1051 (2018) 1–23.
[18] L.I. Hernandez, I. Machado, T. Schafer, F.J. Hernandez, Aptamers overview: selection, features and applications, Curr. Top. Med. Chem. 15 (2015) 1066–1081.
[19] Y. Zhang, D.R. Prakash, H. Zhang, Nucleotide aptamers as theranostic biomaterials, in: W. Cui, X. Zhao (Eds.), Theranostic Bionanomaterials, Elsevier, 2019, pp. 423–446.
[20] M. Oguzhan Caglayan, Electrochemical aptasensors for early cancer diagnosis: a review, Curr. Anal. Chem. 13 (2017) 18–30.
[21] C.H. van den Kieboom, S.L. van der Beek, T. Mészáros, R.E. Gyurcsányi, G. Ferwerda, M.I. de Jonge, Aptasensors for viral diagnostics, TrAC Trends Anal. Chem. 74 (2015) 58–67.
[22] M. Smietana, W.J. Bock, P. Mikulic, A. Ng, R. Chinnappan, M. Zourob, Detection of bacteria using bacteriophages as recognition elements immobilized on long-period fiber gratings, Opt. Express 19 (2011) 7971–7978.
[23] O. Smutok, K. Dmytruk, M. Gonchar, A. Sibirny, W. Schuhmann, Permeabilized cells of flavocytochrome b2 over-producing recombinant yeast *Hansenula polymorpha* as biological recognition element in amperometric lactate biosensors, Biosens. Bioelectron. 23 (2007) 599–605.
[24] W. Cai, K.L. Lesnik, M.J. Wade, E.S. Heidrich, Y. Wang, H. Liu, Incorporating microbial community data with machine learning techniques to predict feed substrates in microbial fuel cells, Biosens. Bioelectron. 133 (2019) 64–71.

[25] J.A. Tanner, Aptamers for malaria diagnosis—a transdisciplinary venture from bio to nano, in: Second International Workshop by the 174th Committee JSPS on Symbiosis of Biology and Nanodevices, 2019, p. 23.
[26] S. Zhao, Aptamer-based microchip electrophoresis assays for amplification detection of carcinoembryonic antigen, in: T.M. Phillips (Ed.), Clinical Applications of Capillary Electrophoresis, Springer, 2019, pp. 251–259.
[27] C. Zhu, D. Liu, Z. Chen, L. Li, T. You, An ultra-sensitive aptasensor based on carbon nanohorns/gold nanoparticles composites for impedimetric detection of carbendazim at picogram levels, J. Colloid Interface Sci. 546 (2019) 92–100.
[28] M. Adeel, M.M. Rahman, J.-J. Lee, Label-free aptasensor for the detection of cardiac biomarker myoglobin based on gold nanoparticles decorated boron nitride nanosheets, Biosens. Bioelectron. 126 (2019) 143–150.
[29] X. Wang, F. Gao, Y. Gong, G. Liu, Y. Zhang, C. Ding, Electrochemical aptasensor based on conductive supramolecular polymer hydrogels for thrombin detection with high selectivity, Talanta 205 (2019) 120140, https://doi.org/10.1016/j.talanta.2019.120140.
[30] S.C.B. Gopinath, K. Awazu, M. Fujimaki, Waveguide-mode sensors as aptasensors, Sensors 12 (2012) 2136–2151, https://doi.org/10.3390/s120202136.
[31] S.G. Meirinho, L.G. Dias, A.M. Peres, L.R. Rodrigues, Voltammetric aptasensors for protein disease biomarkers detection: a review, Biotechnol. Adv. 34 (2016) 941–953.
[32] J.I.A. Rashid, N.A. Yusof, The strategies of DNA immobilization and hybridization detection mechanism in the construction of electrochemical DNA sensor: a review, Sens. Biosensing Res. 16 (2017) 19–31, https://doi.org/10.1016/j.sbsr.2017.09.001.
[33] L. Zhou, M.-H. Wang, J.-P. Wang, Z.-Z. Ye, Application of biosensor surface immobilization methods for aptamer, Chin. J. Anal. Chem. 39 (2011) 432–438, https://doi.org/10.1016/S1872-2040(10)60429-X.
[34] M. Liss, B. Petersen, H. Wolf, E. Prohaska, An aptamer-based quartz crystal protein biosensor, Anal. Chem. 74 (2002) 4488–4495.
[35] X. Wang, P. Dong, W. Yun, Y. Xu, P. He, Y. Fang, A solid-state electrochemiluminescence biosensing switch for detection of thrombin based on ferrocene-labeled molecular beacon aptamer, Biosens. Bioelectron. 24 (2009) 3288–3292, https://doi.org/10.1016/j.bios.2009.04.019.
[36] H. Ceretti, B. Ponce, S.A. Ramírez, J.M. Montserrat, Adenosine reagentless electrochemical aptasensor using a phosphorothioate immobilization strategy, Electroanalysis 22 (2010) 147–150.
[37] Y. Shevchenko, T.J. Francis, D.A.D. Blair, R. Walsh, M.C. DeRosa, J. Albert, In situ biosensing with a surface plasmon resonance fiber grating aptasensor, Anal. Chem. 83 (2011) 7027–7034.
[38] L. Wang, C. Zhu, L. Han, L. Jin, M. Zhou, S. Dong, Label-free, regenerative and sensitive surface plasmon resonance and electrochemical aptasensors based on graphene, Chem. Commun. 47 (2011) 7794–7796.
[39] Z.-M. Dong, G.-C. Zhao, Quartz crystal microbalance aptasensor for sensitive detection of mercury (II) based on signal amplification with gold nanoparticles, Sensors 12 (2012) 7080–7094.
[40] R. Wang, L. Wang, Z.T. Callaway, H. Lu, T.J. Huang, Y. Li, A nanowell-based QCM aptasensor for rapid and sensitive detection of avian influenza virus, Sens. Actuators B 240 (2017) 934–940, https://doi.org/10.1016/j.snb.2016.09.067.
[41] L. Zhou, J. Wang, D. Li, Y. Li, An electrochemical aptasensor based on gold nanoparticles dotted graphene modified glassy carbon electrode for label-free detection of bisphenol A in milk samples, Food Chem. 162 (2014) 34–40, https://doi.org/10.1016/j.foodchem.2014.04.058.

[42] X. Li, H. Qi, L. Shen, Q. Gao, C. Zhang, Electrochemical aptasensor for the determination of cocaine incorporating gold nanoparticles modification, Electroanalysis 20 (2008) 1475–1482.
[43] X. Xu, C. Xu, Y. Ying, Aptasensor for the simple detection of ochratoxin A based on side-by-side assembly of gold nanorods, RSC Adv. 6 (2016) 50437–50443.
[44] S. Dai, S. Wu, N. Duan, Z. Wang, A luminescence resonance energy transfer based aptasensor for the mycotoxin Ochratoxin A using upconversion nanoparticles and gold nanorods, Microchim. Acta. 183 (2016) 1909–1916.
[45] V. Mazzaracchio, D. Neagu, A. Porchetta, E. Marcoccio, A. Pomponi, G. Faggioni, N. D'Amore, A. Notargiacomo, M. Pea, D. Moscone, A label-free impedimetric aptasensor for the detection of *Bacillus anthracis* spore simulant, Biosens. Bioelectron. 126 (2019) 640–646.
[46] A. Florea, Z. Taleat, C. Cristea, M. Mazloum-Ardakani, R. Săndulescu, Label free MUC1 aptasensors based on electrodeposition of gold nanoparticles on screen printed electrodes, Electrochem. Commun. 33 (2013) 127–130, https://doi.org/10.1016/j.elecom.2013.05.008.
[47] A. Blidar, B. Feier, M. Tertis, R. Galatus, C. Cristea, Electrochemical surface plasmon resonance (EC-SPR) aptasensor for ampicillin detection, Anal. Bioanal. Chem. 411 (2019) 1053–1065.
[48] H.-M. So, K. Won, Y.H. Kim, B.-K. Kim, B.H. Ryu, P.S. Na, H. Kim, J.-O. Lee, Single-walled carbon nanotube biosensors using aptamers as molecular recognition elements, J. Am. Chem. Soc. 127 (2005) 11906–11907.
[49] D.O. Demirkol, S. Timur, A sandwich-type assay based on quantum dot/aptamer bioconjugates for analysis of *E. coli* O157:H7 in microtiter plate format, Int. J. Polym. Mater. Polym. Biomater. 65 (2016) 85–90.
[50] N. Paniel, J. Baudart, A. Hayat, L. Barthelmebs, Aptasensor and genosensor methods for detection of microbes in real world samples, Methods 64 (2013) 229–240.
[51] T.T. Nguyen, K.L. Sly, J.C. Conboy, Comparison of the energetics of avidin, streptavidin, neutrAvidin, and anti-biotin antibody binding to biotinylated lipid bilayer examined by second-harmonic generation, Anal. Chem. 84 (2011) 201–208.
[52] Y.-J. Kim, Y.S. Kim, J.H. Niazi, M.B. Gu, Electrochemical aptasensor for tetracycline detection, Bioprocess Biosyst. Eng. 33 (2010) 31.
[53] J. Yoon, N. Choi, J. Ko, K. Kim, S. Lee, J. Choo, Highly sensitive detection of thrombin using SERS-based magnetic aptasensors, Biosens. Bioelectron. 47 (2013) 62–67.
[54] A. Porfirieva, G. Evtugyn, T. Hianik, Polyphenothiazine modified electrochemical aptasensor for detection of human α-thrombin, Electroanalysis 19 (2007) 1915–1920.
[55] L. Chen, L. Sha, Y. Qiu, G. Wang, H. Jiang, X. Zhang, An amplified electrochemical aptasensor based on hybridization chain reactions and catalysis of silver nanoclusters, Nanoscale 7 (2015) 3300–3308.
[56] W. Zhao, W. Chiuman, M.A. Brook, Y. Li, Simple and rapid colorimetric biosensors based on DNA aptamer and noncrosslinking gold nanoparticle aggregation, Chembiochem 8 (2007) 727–731.
[57] G.Q. Wang, L.X. Chen, Aptameric SERS sensor for Hg2+ analysis using silver nanoparticles, Chin. Chem. Lett. 20 (2009) 1475–1477.
[58] H. Cho, B.R. Baker, S. Wachsmann-Hogiu, C.V. Pagba, T.A. Laurence, S.M. Lane, L.P. Lee, J.B.-H. Tok, Aptamer-based SERRS sensor for thrombin detection, Nano Lett. 8 (2008) 4386–4390.
[59] S. Nath, S.K. Ghosh, S. Kundu, S. Praharaj, S. Panigrahi, T. Pal, Is gold really softer than silver? HSAB principle revisited, J. Nanopart. Res. 8 (2006) 111–116.
[60] S. Su, R. Nutiu, C.D.M. Filipe, Y. Li, R. Pelton, Adsorption and covalent coupling of ATP-binding DNA aptamers onto cellulose, Langmuir 23 (2007) 1300–1302.

[61] Y. Liu, K. Yan, O.K. Okoth, J. Zhang, A label-free photoelectrochemical aptasensor based on nitrogen-doped graphene quantum dots for chloramphenicol determination, Biosens. Bioelectron. 74 (2015) 1016–1021.
[62] A.K.H. Cheng, D. Sen, H.-Z. Yu, Design and testing of aptamer-based electrochemical biosensors for proteins and small molecules, Bioelectrochemistry 77 (2009) 1–12.
[63] K. Han, Z. Liang, N. Zhou, Design strategies for aptamer-based biosensors, Sensors 10 (2010) 4541–4557.
[64] J.R. Lomeda, C.D. Doyle, D.V. Kosynkin, W.-F. Hwang, J.M. Tour, Diazonium functionalization of surfactant-wrapped chemically converted graphene sheets, J. Am. Chem. Soc. 130 (2008) 16201–16206.
[65] L. Bai, R. Yuan, Y. Chai, Y. Yuan, Y. Zhuo, L. Mao, Bi-enzyme functionlized hollow PtCo nanochains as labels for an electrochemical aptasensor, Biosens. Bioelectron. 26 (2011) 4331–4336.
[66] B. Zhang, H. Ding, Q. Chen, T. Wang, K. Zhang, Prussian blue nanoparticle-labeled aptasensing platform on graphene oxide for voltammetric detection of α-fetoprotein in hepatocellular carcinoma with target recycling, Analyst 144 (2019) 4858–4864.
[67] H. Ehzari, M. Safari, M. Shahlaei, A signal amplification by QDs used for ferrocene-labeled sandwich aptasensor for determination of Hg2+ in water samples, J. Iran. Chem. Soc. 16 (2019) 2555–2564.
[68] G.B. Aktas, V. Skouridou, L. Masip, Sandwich-type aptasensor employing modified aptamers and enzyme-DNA binding protein conjugates, Anal. Bioanal. Chem. 411 (2019) 3581–3589.
[69] H.-M. Wang, Y. Fang, P.-X. Yuan, A.-J. Wang, X. Luo, J.-J. Feng, Construction of ultrasensitive label-free aptasensor for thrombin detection using palladium nanocones boosted electrochemiluminescence system, Electrochim. Acta 310 (2019) 195–202.
[70] R. Khan, S. Ben Aissa, T.A. Sherazi, G. Catanante, A. Hayat, J.L. Marty, Development of an impedimetric aptasensor for label free detection of patulin in apple juice, Molecules 24 (2019) 1017.
[71] S. Yan, X. Lai, Y. Wang, N. Ye, Y. Xiang, Label free aptasensor for ultrasensitive detection of tobramycin residue in pasteurized cow's milk based on resonance scattering spectra and nanogold catalytic amplification, Food Chem. 295 (2019) 36–41.
[72] Y. Feng, T. Yan, T. Wu, N. Zhang, Q. Yang, M. Sun, L. Yan, B. Du, Q. Wei, A label-free photoelectrochemical aptasensing platform base on plasmon Au coupling with MOF-derived In_2O_3@g-C_3N_4 nanoarchitectures for tetracycline detection, Sens. Actuators B 298 (2019) 126817.
[73] Z. Saberi, B. Rezaei, A.A. Ensafi, Fluorometric label-free aptasensor for detection of the pesticide acetamiprid by using cationic carbon dots prepared with cetrimonium bromide, Microchim. Acta. 186 (2019) 273.
[74] S. Lei, L. Xu, Z. Liu, L. Zou, G. Li, B. Ye, An enzyme-free and label-free signal-on aptasensor based on DNAzyme-driven DNA walker strategy, Anal. Chim. Acta 1081 (2019) 59–64.
[75] Y. Zhu, K. Yan, Z. Xu, J. Wu, J. Zhang, Cathodic "signal-on" photoelectrochemical aptasensor for chloramphenicol detection using hierarchical porous flower-like Bi-BiOI@C composite, Biosens. Bioelectron. 131 (2019) 79–87.
[76] H. Zhang, F. Luo, P. Wang, L. Guo, B. Qiu, Z. Lin, Signal-on electrochemiluminescence aptasensor for bisphenol A based on hybridization chain reaction and electrically heated electrode, Biosens. Bioelectron. 129 (2019) 36–41.
[77] X. Qiao, F. Xia, D. Tian, P. Chen, J. Liu, J. Gu, C. Zhou, Ultrasensitive "signal-on" electrochemical aptasensor for assay of acetamiprid residues based on copper-centered metal-organic frameworks, Anal. Chim. Acta 1050 (2019) 51–59.

[78] L. Yang, X. Zhong, L. Huang, H. Deng, R. Yuan, Y. Yua, C_{60}@C_3N_4 nanocomposites as quencher for signal-off photoelectrochemical aptasensor with Au nanoparticle decorated perylene tetracarboxylic acid as platform, Anal. Chim. Acta 1077 (2019) 281–287.
[79] W. Chen, M. Zhu, Q. Liu, Y. Guo, H. Wang, K. Wang, Fabricating photoelectrochemical aptasensor for sensitive detection of aflatoxin B1 with visible-light-driven BiOBr/nitrogen-doped graphene nanoribbons, J. Electroanal. Chem. 840 (2019) 67–73.
[80] C. Sun, M. Liu, H. Sun, H. Lu, G. Zhao, Immobilization-free photoelectrochemical aptasensor for environmental pollutants: design, fabrication and mechanism, Biosens. Bioelectron. 140 (2019) 111352.
[81] L. Xu, Z. Liu, S. Lei, D. Huang, L. Zou, B. Ye, A sandwich-type electrochemical aptasensor for the carcinoembryonic antigen via biocatalytic precipitation amplification and by using gold nanoparticle composites, Microchim. Acta. 186 (2019) 473.
[82] S. Liu, Y. Wang, W. Xu, X. Leng, H. Wang, Y. Guo, J. Huang, A novel sandwich-type electrochemical aptasensor based on GR-3D Au and aptamer-AuNPs-HRP for sensitive detection of oxytetracycline, Biosens. Bioelectron. 88 (2017) 181–187.
[83] J.-J. Zhang, J.-T. Cao, G.-F. Shi, K.-J. Huang, Y.-M. Liu, S.-W. Ren, A luminol electrochemiluminescence aptasensor based on glucose oxidase modified gold nanoparticles for measurement of platelet-derived growth factor BB, Talanta 132 (2015) 65–71.
[84] A. Ravalli, L. Rivas, A. De La Escosura-Muñiz, J. Pons, A. Merkoçi, G. Marrazza, A DNA aptasensor for electrochemical detection of vascular endothelial growth factor, J. Nanosci. Nanotechnol. 15 (2015) 3411–3416.
[85] Y.H. Tennico, D. Hutanu, M.T. Koesdjojo, C.M. Bartel, V.T. Remcho, On-chip aptamer-based sandwich assay for thrombin detection employing magnetic beads and quantum dots, Anal. Chem. 82 (2010) 5591–5597.
[86] A. Abbaspour, F. Norouz-Sarvestani, A. Noori, N. Soltani, Aptamer-conjugated silver nanoparticles for electrochemical dual-aptamer-based sandwich detection of *Staphylococcus aureus*, Biosens. Bioelectron. 68 (2015) 149–155.
[87] P. Jing, H. Yi, S. Xue, Y. Chai, R. Yuan, W. Xu, A sensitive electrochemical aptasensor based on palladium nanoparticles decorated graphene–molybdenum disulfide flower-like nanocomposites and enzymatic signal amplification, Anal. Chim. Acta 853 (2015) 234–241.
[88] S. Pilehvar, K. De Wael, Recent advances in electrochemical biosensors based on fullerene-C60 nano-structured platforms, Biosensors 5 (2015) 712–735.
[89] T. Li, E. Wang, S. Dong, Chemiluminescence thrombin aptasensor using high-activity DNAzyme as catalytic label, Chem. Commun. 43 (2008) 5520–5522.
[90] W. Xu, Y. Wang, S. Liu, J. Yu, H. Wang, J. Huang, A novel sandwich-type electrochemical aptasensor for sensitive detection of kanamycin based on GR–PANI and PAMAM–Au nanocomposites, New J. Chem. 38 (2014) 4931–4937.
[91] G.-F. Shi, J.-T. Cao, J.-J. Zhang, Y.-M. Liu, Y.-H. Chen, S.-W. Ren, An electrochemiluminescence aptasensor based on flowerlike CdS–MoS_2 composites and DNAzyme for detection of immunoglobulin E, Sens. Actuators B 220 (2015) 340–346.
[92] Y. Liu, N. Tuleouva, E. Ramanculov, A. Revzin, Aptamer-based electrochemical biosensor for interferon gamma detection, Anal. Chem. 82 (2010) 8131–8136.
[93] M. Roushani, F. Shahdost-fard, A novel ultrasensitive aptasensor based on silver nanoparticles measured via enhanced voltammetric response of electrochemical reduction of riboflavin as redox probe for cocaine detection, Sens. Actuators B 207 (2015) 764–771.

[94] Y. Yang, Y. Cen, W.-J. Deng, R.-Q. Yu, T.-T. Chen, X. Chu, An aptasensor based on cobalt oxyhydroxide nanosheets for the detection of thrombin, Anal. Methods 8 (2016) 7199–7203.
[95] Z. Taleat, C. Cristea, G. Marrazza, M. Mazloum-Ardakani, R. Săndulescu, Electrochemical immunoassay based on aptamer–protein interaction and functionalized polymer for cancer biomarker detection, J. Electroanal. Chem. 717–718 (2014) 119–124. https://doi.org/10.1016/j.jelechem.2014.01.015.
[96] M. Mazloum-Ardakani, L. Hosseinzadeh, A sensitive electrochemical aptasensor for TNF-α based on bimetallic Ag@Pt core-shell nanoparticle functionalized graphene nanostructures as labels for signal amplification, J. Electrochem. Soc. 163 (2016) B119–B124.
[97] Y.-M. Liu, M. Zhou, Y.-Y. Liu, K.-J. Huang, J.-T. Cao, J.-J. Zhang, G.-F. Shi, Y.-H. Chen, A novel sandwich electrochemiluminescence aptasensor based on molybdenum disulfide nanosheet–graphene composites and Au nanoparticles for signal amplification, Anal. Methods 6 (2014) 4152–4157.
[98] Y. Chen, X. Liu, S. Guo, J. Cao, J. Zhou, J. Zuo, L. Bai, A sandwich-type electrochemical aptasensor for *Mycobacterium tuberculosis* MPT64 antigen detection using C_{60}NPs decorated N-CNTs/GO nanocomposite coupled with conductive PEI-functionalized metal-organic framework, Biomaterials 216 (2019) 119253.
[99] Y. Zhang, G. Figueroa-Miranda, Z. Lyu, C. Zafiu, D. Willbold, A. Offenhäusser, D. Mayer, Monitoring amyloid-β proteins aggregation based on label-free aptasensor, Sens. Actuators B 288 (2019) 535–542.
[100] Y. Jia, F. Wu, P. Liu, G. Zhou, B. Yu, X. Lou, F. Xia, A label-free fluorescent aptasensor for the detection of Aflatoxin B1 in food samples using AIEgens and graphene oxide, Talanta 198 (2019) 71–77.
[101] X. Zhang, T. Yan, T. Wu, Y. Feng, M. Sun, L. Yan, B. Du, Q. Wei, Fabrication of hierarchical MIL-68(In)-NH_2/MWCNT/CdS composites for constructing label-free photoelectrochemical tetracycline aptasensor platform, Biosens. Bioelectron. 135 (2019) 88–94.
[102] M. Tertis, P.I. Leva, D. Bogdan, M. Suciu, F. Graur, C. Cristea, Impedimetric aptasensor for the label-free and selective detection of Interleukin-6 for colorectal cancer screening, Biosens. Bioelectron. 137 (2019) 123–132.
[103] T. Lee, G.H. Kim, S.M. Kim, K. Hong, Y. Kim, C. Park, H. Sohn, J. Min, Label-free localized surface plasmon resonance biosensor composed of multi-functional DNA 3 way junction on hollow Au spike-like nanoparticles (HAuSN) for avian influenza virus detection, Colloids Surf. B Biointerfaces 182 (2019) 110341.
[104] S.G. Meirinho, L.G. Dias, A.M. Peres, L.R. Rodrigues, Development of an electrochemical RNA-aptasensor to detect human osteopontin, Biosens. Bioelectron. 71 (2015) 332–341.
[105] B. Zhou, X.-Y. Yang, Y.-S. Wang, J.-C. Yi, Z. Zeng, H. Zhang, Y.-T. Chen, X.-J. Hu, Q.-L. Suo, Label-free fluorescent aptasensor of Cd^{2+} detection based on the conformational switching of aptamer probe and SYBR green I, Microchem. J. 144 (2019) 377–382.
[106] M. Mazloum-Ardakani, L. Hosseinzadeh, Z. Taleat, Synthesis and electrocatalytic effect of Ag@Pt core–shell nanoparticles supported on reduced graphene oxide for sensitive and simple label-free electrochemical aptasensor, Biosens. Bioelectron. 74 (2015) 30–36.
[107] H. Li, S. Song, M. Wen, T. Bao, Z. Wu, H. Xiong, X. Zhang, W. Wen, S. Wang, A novel label-free electrochemical impedance aptasensor for highly sensitive detection of human interferon-gamma based on target-induced exonuclease inhibition, Biosens. Bioelectron. 142 (2019) 111532.
[108] L. Alfonta, A. Bardea, O. Khersonsky, E. Katz, I. Willner, Chronopotentiometry and Faradaic impedance spectroscopy as signal transduction methods for the biocatalytic

precipitation of an insoluble product on electrode supports: routes for enzyme sensors, immunosensors and DNA sensors, Biosens. Bioelectron. 16 (2001) 675–687.
[109] L. Feng, Y. Chen, J. Ren, X. Qu, A graphene functionalized electrochemical aptasensor for selective label-free detection of cancer cells, Biomaterials 32 (2011) 2930–2937.
[110] B. Bohunicky, S.A. Mousa, Biosensors: the new wave in cancer diagnosis, Nanotechnol. Sci. Appl. 4 (2011) 1.
[111] M. Mazloum-Ardakani, L. Hosseinzadeh, A. Khoshroo, Ultrasensitive electrochemical immunosensor for detection of tumor necrosis factor-α based on functionalized MWCNT-gold nanoparticle/ionic liquid nanocomposite, Electroanalysis 27 (2015) 2518–2526.
[112] M. Mazloum-Ardakani, L. Hosseinzadeh, Highly-sensitive label-free immunosensor for tumor necrosis factor α based on Ag@Pt core–shell nanoparticles supported on MWCNTs as an efficient electrocatalyst nanocomposite, RSC Adv. 5 (2015) 70781–70786.
[113] A. Rahi, N. Sattarahmady, H. Heli, Label-free electrochemical aptasensing of the human prostate-specific antigen using gold nanospears, Talanta 156 (2016) 218–224.
[114] A. Raouafi, A. Sánchez, N. Raouafi, R. Villalonga, Electrochemical aptamer-based bioplatform for ultrasensitive detection of prostate specific antigen, Sens. Actuators B 297 (2019) 126762.
[115] R. Hu, W. Wen, Q. Wang, H. Xiong, X. Zhang, H. Gu, S. Wang, Novel electrochemical aptamer biosensor based on an enzyme–gold nanoparticle dual label for the ultrasensitive detection of epithelial tumour marker MUC1, Biosens. Bioelectron. 53 (2014) 384–389, https://doi.org/10.1016/j.bios.2013.10.015.
[116] Z. Hatami, F. Jalali, M. Amouzadeh Tabrizi, M. Shamsipur, Application of metal-organic framework as redox probe in an electrochemical aptasensor for sensitive detection of MUC1, Biosens. Bioelectron. 141 (2019) 111433, https://doi.org/10.1016/j.bios.2019.111433.
[117] X. Liu, Y. Qin, C. Deng, J. Xiang, Y. Li, A simple and sensitive impedimetric aptasensor for the detection of tumor markers based on gold nanoparticles signal amplification, Talanta 132 (2015) 150–154. https://doi.org/10.1016/j.talanta.2014.08.072.
[118] Y. He, Y. Lin, H. Tang, D. Pang, A graphene oxide-based fluorescent aptasensor for the turn-on detection of epithelial tumor marker mucin 1, Nanoscale 4 (2012) 2054–2059, https://doi.org/10.1039/C2NR12061E.
[119] P. Jolly, N. Formisano, J. Tkáč, P. Kasák, C.G. Frost, P. Estrela, Label-free impedimetric aptasensor with antifouling surface chemistry: a prostate specific antigen case study, Sens. Actuators B 209 (2015) 306–312. https://doi.org/10.1016/j.snb.2014.11.083.
[120] K. Yang, Y. Hu, N. Dong, G. Zhu, T. Zhu, N. Jiang, A novel SERS-based magnetic aptasensor for prostate specific antigen assay with high sensitivity, Biosens. Bioelectron. 94 (2017) 286–291, https://doi.org/10.1016/j.bios.2017.02.048.
[121] W. Argoubi, A. Sánchez, C. Parrado, N. Raouafi, R. Villalonga, Label-free electrochemical aptasensing platform based on mesoporous silica thin film for the detection of prostate specific antigen, Sens. Actuators B 255 (2018) 309–315, https://doi.org/10.1016/j.snb.2017.08.045.
[122] R.-M. Kong, X. Zhang, L. Ding, D. Yang, F. Qu, Label-free fluorescence turn-on aptasensor for prostate-specific antigen sensing based on aggregation-induced emission–silica nanospheres, Anal. Bioanal. Chem. 409 (2017) 5757–5765.

[123] Z. Zhang, W. Yang, J. Wang, C. Yang, F. Yang, X. Yang, A sensitive impedimetric thrombin aptasensor based on polyamidoamine dendrimer, Talanta 78 (2009) 1240–1245, https://doi.org/10.1016/j.talanta.2009.01.034.
[124] X. Wang, J. Zhou, W. Yun, S. Xiao, Z. Chang, P. He, Y. Fang, Detection of thrombin using electrogenerated chemiluminescence based on $Ru(bpy)_3^{2+}$-doped silica nanoparticle aptasensor via target protein-induced strand displacement, Anal. Chim. Acta 598 (2007) 242–248, https://doi.org/10.1016/j.aca.2007.07.050.
[125] C. Zhu, W. Zhu, L. Xu, X. Zhou, A label-free electrochemical aptasensor based on magnetic biocomposites with Pb^{2+}-dependent DNAzyme for the detection of thrombin, Anal. Chim. Acta 1047 (2019) 21–27, https://doi.org/10.1016/j.aca.2018.09.040.
[126] G. Yan, Y. Wang, X. He, K. Wang, J. Liu, Y. Du, A highly sensitive label-free electrochemical aptasensor for interferon-gamma detection based on graphene controlled assembly and nuclease cleavage-assisted target recycling amplification, Biosens. Bioelectron. 44 (2013) 57–63, https://doi.org/10.1016/j.bios.2013.01.010.
[127] T.-L. Chuang, C.-C. Chang, Y. Chu-Su, S.-C. Wei, X. Zhao, P.-R. Hsueh, C.-W. Lin, Disposable surface plasmon resonance aptasensor with membrane-based sample handling design for quantitative interferon-gamma detection, Lab Chip 14 (2014) 2968–2977.
[128] S. Ding, C. Mosher, X.Y. Lee, S.R. Das, A.A. Cargill, X. Tang, B. Chen, E.S. McLamore, C. Gomes, J.M. Hostetter, Rapid and label-free detection of interferon gamma via an electrochemical aptasensor comprising a ternary surface monolayer on a gold interdigitated electrode array, ACS Sens. 2 (2017) 210–217.
[129] K. Hu, J. Liu, J. Chen, Y. Huang, S. Zhao, J. Tian, G. Zhang, An amplified graphene oxide-based fluorescence aptasensor based on target-triggered aptamer hairpin switch and strand-displacement polymerization recycling for bioassays, Biosens. Bioelectron. 42 (2013) 598–602, https://doi.org/10.1016/j.bios.2012.11.025.
[130] Y. Chai, X. Li, M. Yang, Aptamer based determination of the cancer biomarker HER2 by using phosphate-functionalized MnO_2 nanosheets as the electrochemical probe, Microchim. Acta. 186 (2019) 316.
[131] L. Chun, S.-E. Kim, M. Cho, W. Choe, J. Nam, D.W. Lee, Y. Lee, Electrochemical detection of HER2 using single stranded DNA aptamer modified gold nanoparticles electrode, Sens. Actuators B 186 (2013) 446–450, https://doi.org/10.1016/j.snb.2013.06.046.
[132] G. Bezerra, C. Córdula, D. Campos, G. Nascimento, N. Oliveira, M.A. Seabra, V. Visani, S. Lucas, I. Lopes, J. Santos, Electrochemical aptasensor for the detection of HER2 in human serum to assist in the diagnosis of early stage breast cancer, Anal. Bioanal. Chem. 411 (2019) 6667–6676.
[133] S. Yang, M. You, F. Zhang, Q. Wang, P. He, A sensitive electrochemical aptasensing platform based on exonuclease recycling amplification and host-guest recognition for detection of breast cancer biomarker HER2, Sens. Actuators B 258 (2018) 796–802, https://doi.org/10.1016/j.snb.2017.11.119.
[134] D.T. Tran, V. Vermeeren, L. Grieten, S. Wenmackers, P. Wagner, J. Pollet, K.P.F. Janssen, L. Michiels, J. Lammertyn, Nanocrystalline diamond impedimetric aptasensor for the label-free detection of human IgE, Biosens. Bioelectron. 26 (2011) 2987–2993, https://doi.org/10.1016/j.bios.2010.11.053.
[135] S. Khezrian, A. Salimi, H. Teymourian, R. Hallaj, Label-free electrochemical IgE aptasensor based on covalent attachment of aptamer onto multiwalled carbon nanotubes/ionic liquid/chitosan nanocomposite modified electrode, Biosens. Bioelectron. 43 (2013) 218–225, https://doi.org/10.1016/j.bios.2012.12.006.

[136] C.-Y. Lee, K.-Y. Wu, H.-L. Su, H.-Y. Hung, Y.-Z. Hsieh, Sensitive label-free electrochemical analysis of human IgE using an aptasensor with cDNA amplification, Biosens. Bioelectron. 39 (2013) 133–138, https://doi.org/10.1016/j.bios.2012.07.009.
[137] C.-C. Chang, C.-Y. Chen, X. Zhao, T.-H. Wu, S.-C. Wei, C.-W. Lin, Label-free colorimetric aptasensor for IgE using DNA pseudoknot probe, Analyst 139 (2014) 3347–3351.
[138] Y. Liu, Q. Zhou, A. Revzin, An aptasensor for electrochemical detection of tumor necrosis factor in human blood, Analyst 138 (2013) 4321–4326, https://doi.org/10.1039/C3AN00818E.
[139] L. Liu, F. Liu, D. Jiang, G. Xiang, C. Liu, J. Yang, X. Pu, Hybridization chain reaction and target recycling enhanced tumor necrosis factor alpha aptasensor with host-guest interaction for signal probe collection, Sens. Actuators B 231 (2016) 680–687, https://doi.org/10.1016/j.snb.2016.03.098.
[140] M.H. Ghalehno, M. Mirzaei, M. Torkzadeh-Mahani, Aptamer-based determination of tumor necrosis factor α using a screen-printed graphite electrode modified with gold hexacyanoferrate, Microchim. Acta. 185 (2018) 165.
[141] M.H. Ghalehno, M. Mirzaei, M. Torkzadeh-Mahani, Electrochemical aptasensor for tumor necrosis factor α using aptamer–antibody sandwich structure and cobalt hexacyanoferrate for signal amplification, J. Iran. Chem. Soc. 16 (2019) 1783–1791.
[142] Z. Wu, H. Li, Z. Liu, An aptasensor for carcinoembryonic antigen based on upconversion fluorescence resonance energy transfer, Sens. Actuators B 206 (2015) 531–537, https://doi.org/10.1016/j.snb.2014.09.084.
[143] Z. Si, B. Xie, Z. Chen, C. Tang, T. Li, M. Yang, Electrochemical aptasensor for the cancer biomarker CEA based on aptamer induced current due to formation of molybdophosphate, Microchim. Acta. 184 (2017) 3215–3221.
[144] J.-Y. Huang, L. Zhao, W. Lei, W. Wen, Y.-J. Wang, T. Bao, H.-Y. Xiong, X.-H. Zhang, S.-F. Wang, A high-sensitivity electrochemical aptasensor of carcinoembryonic antigen based on graphene quantum dots-ionic liquid-nafion nanomatrix and DNAzyme-assisted signal amplification strategy, Biosens. Bioelectron. 99 (2018) 28–33, https://doi.org/10.1016/j.bios.2017.07.036.
[145] S. Riethdorf, H. Fritsche, V. Müller, T. Rau, C. Schindlbeck, B. Rack, W. Janni, C. Coith, K. Beck, F. Jänicke, Detection of circulating tumor cells in peripheral blood of patients with metastatic breast cancer: a validation study of the CellSearch system, Clin. Cancer Res. 13 (2007) 920–928.
[146] K. Pantel, R.H. Brakenhoff, Dissecting the metastatic cascade, Nat. Rev. Cancer 4 (2004) 448.
[147] S.A. Hosseini, M. Abdolahad, S. Zanganeh, M. Dahmardeh, M. Gharooni, H. Abiri, A. Alikhani, S. Mohajerzadeh, O. Mashinchian, Nanoelectromechanical chip (NELMEC) combination of nanoelectronics and microfluidics to diagnose epithelial and mesenchymal circulating tumor cells from leukocytes, Small 12 (2016) 883–891.
[148] M. Maltez-da Costa, A. de la Escosura-Muñiz, C. Nogués, L. Barrios, E. Ibáñez, A. Merkoçi, Simple monitoring of cancer cells using nanoparticles, Nano Lett. 12 (2012) 4164–4171.
[149] L. Kashefi-Kheyrabadi, M.A. Mehrgardi, E. Wiechec, A.P.F. Turner, A. Tiwari, Ultrasensitive detection of human liver hepatocellular carcinoma cells using a label-free aptasensor, Anal. Chem. 86 (2014) 4956–4960.
[150] L. Cerchia, V. De Franciscis, Targeting cancer cells with nucleic acid aptamers, Trends Biotechnol. 28 (2010) 517–525.
[151] B. Hong, Y. Zu, Detecting circulating tumor cells: current challenges and new trends, Theranostics 3 (2013) 377.

[152] M. Yan, G. Sun, F. Liu, J. Lu, J. Yu, X. Song, An aptasensor for sensitive detection of human breast cancer cells by using porous GO/Au composites and porous PtFe alloy as effective sensing platform and signal amplification labels, Anal. Chim. Acta 798 (2013) 33–39.
[153] S. Cai, M. Chen, M. Liu, W. He, Z. Liu, D. Wu, Y. Xia, H. Yang, J. Chen, A signal amplification electrochemical aptasensor for the detection of breast cancer cell via free-running DNA walker, Biosens. Bioelectron. 85 (2016) 184–189.
[154] Y.-S. Borghei, M. Hosseini, M. Dadmehr, S. Hosseinkhani, M.R. Ganjali, R. Sheikhnejad, Visual detection of cancer cells by colorimetric aptasensor based on aggregation of gold nanoparticles induced by DNA hybridization, Anal. Chim. Acta 904 (2016) 92–97, https://doi.org/10.1016/j.aca.2015.11.026.
[155] N. Liu, J. Song, Y. Lu, J.J. Davis, F. Gao, X. Luo, Electrochemical aptasensor for ultra-low fouling cancer cell quantification in complex biological media based on designed branched peptides, Anal. Chem. 91 (2019) 8334–8340, https://doi.org/10.1021/acs.analchem.9b01129.
[156] S. Wu, N. Yang, L. Zhong, Y. Luo, H. Wang, W. Gong, S. Zhou, Y. Li, J. He, H. Cao, A novel label-free terbium (III)-aptamer based aptasensor for ultrasensitive and highly specific detection of acute lymphoma leukemia cells, Analyst 144 (2019) 3843–3852.
[157] X. Zhang, K. Xiao, L. Cheng, H. Chen, B. Liu, S. Zhang, J. Kong, Visual and highly sensitive detection of cancer cells by a colorimetric aptasensor based on cell-triggered cyclic enzymatic signal amplification, Anal. Chem. 86 (2014) 5567–5572.
[158] K. Xiao, J. Liu, H. Chen, S. Zhang, J. Kong, A label-free and high-efficient GO-based aptasensor for cancer cells based on cyclic enzymatic signal amplification, Biosens. Bioelectron. 91 (2017) 76–81, https://doi.org/10.1016/j.bios.2016.11.057.
[159] C.-Y. Lu, J.-J. Xu, Z.-H. Wang, H.-Y. Chen, A novel signal-amplified electrochemical aptasensor based on supersandwich G-quadruplex DNAzyme for highly sensitive cancer cell detection, Electrochem. Commun. 52 (2015) 49–52, https://doi.org/10.1016/j.elecom.2015.01.015.
[160] K. Wang, R. Zhang, N. Sun, X. Li, J. Wang, Y. Cao, R. Pei, Near-infrared light-driven photoelectrochemical aptasensor based on the upconversion nanoparticles and TiO_2/CdTe heterostructure for detection of cancer cells, ACS Appl. Mater. Interfaces 8 (2016) 25834–25839.
[161] X. Hun, H. Chen, W. Wang, A electrogenerated chemiluminescence biosensor for Ramos cancer cell using DNA encapsulated $Ru(bpy)_3Cl_2$ as signal probe, Biosens. Bioelectron. 26 (2011) 3887–3893, https://doi.org/10.1016/j.bios.2011.03.004.
[162] E.V. Koonin, T.G. Senkevich, V.V. Dolja, The ancient virus world and evolution of cells, Biol. Direct 1 (2006) 29.
[163] A. Afzal, A. Mujahid, R. Schirhagl, S. Bajwa, U. Latif, S. Feroz, Gravimetric viral diagnostics: QCM based biosensors for early detection of viruses, Chemosensors 5 (2017) 7.
[164] T. Janas, T. Janas, The selection of aptamers specific for membrane molecular targets, Cell. Mol. Biol. Lett. 16 (2011) 25.
[165] S. Song, L. Wang, J. Li, C. Fan, J. Zhao, Aptamer-based biosensors, TrAC Trends Anal. Chem. 27 (2008) 108–117.
[166] Y. Wang, Y. Wang, B. Liu, Fluorescent detection of ATP based on signaling DNA aptamer attached silica nanoparticles, Nanotechnology 19 (2008) 415605.
[167] M. Labib, A.S. Zamay, D. Muharemagic, A.V. Chechik, J.C. Bell, M.V. Berezovski, Aptamer-based viability impedimetric sensor for viruses, Anal. Chem. 84 (2012) 1813–1816.
[168] S. Karash, R. Wang, L. Kelso, H. Lu, T.J. Huang, Y. Li, Rapid detection of avian influenza virus H5N1 in chicken tracheal samples using an impedance aptasensor with gold nanoparticles for signal amplification, J. Virol. Methods 236 (2016) 147–156, https://doi.org/10.1016/j.jviromet.2016.07.018.

[169] H. Bai, R. Wang, B. Hargis, H. Lu, Y. Li, A SPR aptasensor for detection of avian influenza virus H5N1, Sensors 12 (2012) 12506–12518.
[170] K. Ghanbari, M. Roushani, A. Azadbakht, Ultra-sensitive aptasensor based on a GQD nanocomposite for detection of hepatitis C virus core antigen, Anal. Biochem. 534 (2017) 64–69, https://doi.org/10.1016/j.ab.2017.07.016.
[171] V.I. Kukushkin, N.M. Ivanov, A.A. Novoseltseva, A.S. Gambaryan, I.V. Yaminsky, A.M. Kopylov, E.G. Zavyalova, Highly sensitive detection of influenza virus with SERS aptasensor, PLoS One 14 (2019) e0216247.
[172] D. Cheng, Y. Zhang, D. Wen, Z. Guo, H. Yang, Y. Liu, J. Kong, Hairpin probes based click polymerization for label-free electrochemical detection of human T-lymphotropic virus types II, Anal. Chim. Acta 1059 (2019) 86–93, https://doi.org/10.1016/j.aca.2019.01.027.
[173] N. Duan, S. Wu, J. Wang, Y. Zou, Z. Wang, Quantum dot-based F_0F_1-ATPase aptasensor for *Vibrio parahaemolyticus* detection, Food Anal. Methods 12 (2019) 1849–1857.
[174] M. Vestergaard, K. Kerman, E. Tamiya, An overview of label-free electrochemical protein sensors, Sensors 7 (2007) 3442–3458, https://doi.org/10.3390/s7123442.
[175] G. Liu, D.-Q. Feng, Z. Li, Y. Feng, Target-activatable gold nanoparticle-based aptasensing for protein biomarkers using stimuli-responsive aggregation, Talanta 192 (2019) 112–117.
[176] A. Alawad, G. Istamboulié, C. Calas-Blanchard, T. Noguer, A reagentless aptasensor based on intrinsic aptamer redox activity for the detection of tetracycline in water, Sens. Actuators B 288 (2019) 141–146.
[177] D. Chen, D. Yao, C. Xie, D. Liu, Development of an aptasensor for electrochemical detection of tetracycline, Food Control 42 (2014) 109–115.
[178] H. Ke, M. Liu, L. Zhuang, Z. Li, L. Fan, G. Zhao, A fetomolar level 17β-estradiol electrochemical aptasensor constructed on hierachical dendritic gold modified boron-doped diamond electrode, Electrochim. Acta 137 (2014) 146–153.
[179] K. Abnous, N.M. Danesh, M. Alibolandi, M. Ramezani, S.M. Taghdisi, A.S. Emrani, A novel electrochemical aptasensor for ultrasensitive detection of fluoroquinolones based on single-stranded DNA-binding protein, Sens. Actuators B 240 (2017) 100–106.
[180] J. Dapra, L.H. Lauridsen, A.T. Nielsen, N. Rozlosnik, Comparative study on aptamers as recognition elements for antibiotics in a label-free all-polymer biosensor, Biosens. Bioelectron. 43 (2013) 315–320.
[181] H. Derikvand, M. Roushani, A.R. Abbasi, Z. Derikvand, A. Azadbakht, Design of folding-based impedimetric aptasensor for determination of the nonsteroidal anti-inflammatory drug, Anal. Biochem. 513 (2016) 77–86.
[182] L. Yang, H. Ni, C. Li, X. Zhang, K. Wen, Y. Ke, H. Yang, W. Shi, S. Zhang, J. Shen, Z. Wang, Development of a highly specific chemiluminescence aptasensor for sulfamethazine detection in milk based on in vitro selected aptamers, Sens. Actuators B 281 (2019) 801–811, https://doi.org/10.1016/j.snb.2018.10.143.
[183] X.-X. Chen, Z.-Z. Lin, C.-Y. Hong, H.-P. Zhong, Q.-H. Yao, Z.-Y. Huang, Label-free fluorescence-based aptasensor for the detection of sulfadimethoxine in water and fish, Appl. Spectrosc. 73 (2019) 294–303.
[184] Y. Li, G. Ran, G. Lu, X. Ni, D. Liu, J. Sun, C. Xie, D. Yao, W. Bai, Highly sensitive label-free electrochemical aptasensor based on screen-printed electrode for detection of cadmium (II) ions, J. Electrochem. Soc. 166 (2019) B449–B455.
[185] Y. Wang, G. Zhao, Q. Zhang, H. Wang, Y. Zhang, W. Cao, N. Zhang, B. Du, Q. Wei, Electrochemical aptasensor based on gold modified graphene nanocomposite with different morphologies for ultrasensitive detection of Pb^{2+}, Sens. Actuators B 288 (2019) 325–331, https://doi.org/10.1016/j.snb.2019.03.010.

[186] Q. Wang, X. Qin, L. Geng, Y. Wang, Label-free electrochemical aptasensor for sensitive detection of malachite green based on Au nanoparticle/graphene quantum dots/tungsten disulfide nanocomposites, Nanomaterials 9 (2019) 229.
[187] C. Hongxia, H. Ji, L. Zaijun, L. Ruiyi, Y. Yongqiang, S. Xiulan, Electrochemical aptasensor for detection of acetamiprid in vegetables with graphene aerogel-glutamic acid functionalized graphene quantum dot/gold nanostars as redox probe with catalyst, Sens. Actuators B 298 (2019) 126866.
[188] N. Kaur, A. Bharti, S. Batra, S. Rana, S. Rana, A. Bhalla, N. Prabhakar, An electrochemical aptasensor based on graphene doped chitosan nanocomposites for determination of Ochratoxin A, Microchem. J. 144 (2019) 102–109.
[189] L. Zeng, D. Zhou, J. Gong, C. Liu, J. Chen, Highly sensitive aptasensor for trace arsenic (III) detection using DNAzyme as the biocatalytic amplifier, Anal. Chem. 91 (2019) 1724–1727.
[190] J. Luo, D. Jiang, T. Liu, J. Peng, Z. Chu, W. Jin, High-performance electrochemical mercury aptasensor based on synergistic amplification of Pt nanotube arrays and Fe_3O_4/rGO nanoprobes, Biosens. Bioelectron. 104 (2018) 1–7, https://doi.org/10.1016/j.bios.2017.12.044.
[191] R. Elshafey, M. Siaj, M. Zourob, DNA aptamers selection and characterization for development of label-free impedimetric aptasensor for neurotoxin anatoxin-a, Biosens. Bioelectron. 68 (2015) 295–302.
[192] L. Rivas, C.C. Mayorga-Martinez, D. Quesada-González, A. Zamora-Gálvez, A. de la Escosura-Muñiz, A. Merkoçi, Label-free impedimetric aptasensor for ochratoxin-A detection using iridium oxide nanoparticles, Anal. Chem. 87 (2015) 5167–5172.
[193] Y. Jiao, H. Jia, Y. Guo, H. Zhang, Z. Wang, X. Sun, J. Zhao, An ultrasensitive aptasensor for chlorpyrifos based on ordered mesoporous carbon/ferrocene hybrid multiwalled carbon nanotubes, RSC Adv. 6 (2016) 58541–58548.
[194] N. Prabhakar, H. Thakur, A. Bharti, N. Kaur, Chitosan-iron oxide nanocomposite based electrochemical aptasensor for determination of malathion, Anal. Chim. Acta 939 (2016) 108–116.