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REVIEW

Electrochemical biosensors based on nucleic acid aptamers

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Abstract

During recent decades, nucleic acid aptamers have emerged as powerful biological recognition elements for electrochemical affinity biosensors. These bioreceptors emulate or improve on antibody-based biosensors because of their excellent characteristics as bioreceptors, including limitless selection capacity for a large variety of analytes, easy and cost-effective production, high stability and reproducibility, simple chemical modification, stable and oriented immobilization on electrode surfaces, enhanced target affinity and selectivity, and possibility to design them in target-sensitive 3D folded structures. This review provides an overview of the state of the art of electrochemical aptasensor technology, focusing on novel aptamer-based electroanalytical assay configurations and providing examples to illustrate the different possibilities. Future prospects for this technology are also discussed.

Keywords Aptamer · Biosensor · Electrochemical · Nanomaterials · Nucleic acids

Introduction

Electrochemical biosensors are self-contained integrated receptor–transducer analytical devices based on a biological recognition element incorporated or in close contact with an electrochemical transducer [1]. These sensors exhibit the inherent selectivity and specificity of the biological receptor, combined with the high sensitivity and low detection limit of electroanalytical methods of detection. They can be miniaturized and used with portable instruments, mass-produced at relatively low cost, and provide a fast analytical response with only a few microliters of sample. In addition, these biosensors are simple, user-friendly, cost-effective, and disposable. Accordingly, electrochemical biosensors have been predicted as promising alternatives to traditional analytical instruments [2, 3].

The global market for electrochemical biosensors reached USD 12.8 billion in 2018, mainly fueled by the massive demand for blood glucose monitoring systems. This market is expected to reach USD 23.7 billion by 2022, growing at a compound annual rate of 9.7% according to WiseGuy Reports [4]. Such extraordinary growth is supported by the social and economic demands for new portable analytical devices for personalized medicine and other health care applications, mainly addressed to the point-of-care detection of biomarkers for chronic and emergent diseases such as cancer, cardiovascular diseases, stroke, chronic respiratory diseases, and tuberculosis. Other applications such as food quality control and safety, in situ environmental monitoring, industrial and agricultural production control, homeland security, and biodefense also demand this type of sensor system [5].

Driven by this demand, increased research efforts have been devoted to this field. In this sense, electrochemical biosensor technology has become one of the most extensively studied disciplines in chemistry, with an impressive number of studies published and patents issued during the last decade. Regrettably, this growth is more lateral than progressive, and with only few exceptions, these biosensors have remained constrained to academic demonstrations and have not yet proven to be commercially viable. Low reproducibility and repeatability, limited shelf life and stability of the biorecognition element, and nonspecific analytical response in real samples remain the main barriers for entry of electrochemical biosensors to the market [2].

The most recent advances in electrochemical biosensors have been triggered by the advent of nanotechnology, and the consequent progress in nanomaterials chemistry and engineering, nanolithography, and related areas [6–8]. In addition, the discovery of nucleic acid aptamers as new biological receptors with unique properties has opened new possibilities for affinity-based electrochemical sensors [9, 10].

Dedicated to the memory of our friend Prof. Marco Mascini.

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Aptamers are now largely used at the academic level for the construction of electrochemical biosensors. As illustrated in Fig. 1, the interest in these affinity bioreceptors has continuously growing during the last decade. In 2018, articles related to electrochemical aptasensors represented about 28% of the total number of articles published on electrochemical biosensors, as reported by the Web of Science.

Despite this research effort, electrochemical aptasensors have not yet broken into the market [11]. To our knowledge, several companies, such as Nanogenecs (https://www.nanogenecs.com), CibusDx (https://cibusdx.com), Orion High Technologies (https://www.orion-hitech.com), and InnaMed (https://www.innamed.com), are currently developing portable aptamer-based electrochemical sensing platforms for clinical diagnosis and food safety. These analytical devices could be commercialized in the coming years, creating a huge impact and pull effect in the global biosensor market.

This review summarizes the state of the art of electrochemical aptasensor technology. For this purpose, reports of outstanding significance published during the last 5 years will be classified according to the sensing mechanism used. On this basis, the most relevant advances in aptasensor design and assembly and the moist relevant biosensing approach will be discussed.

Why aptamers?

The term "aptamer," derived from the Latin words *aptus* ("to fit") and *meros* ("particle"), is used to identify short RNA or single-stranded DNA synthetic oligonucleotides, as well as peptides that bind a target molecule with high affinity, selectivity, and specificity [12]. These artificial bioreceptors were independently discovered in 1990 by Ellington and Szostak [13] and Tuerk and Gold [14], who prepared RNA molecules that bind organic dyes and T4 DNA polymerase, respectively.

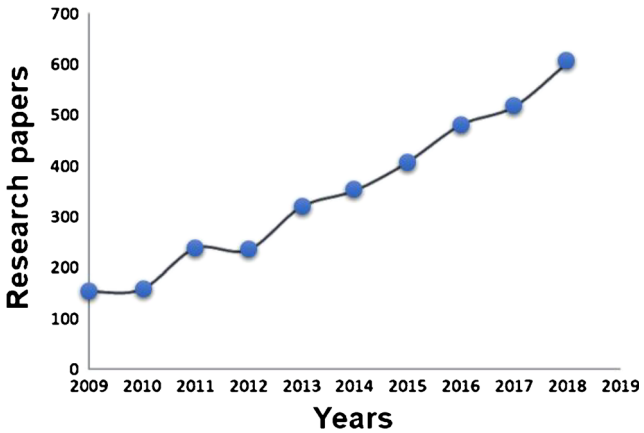

Fig. 1 Research articles published on electrochemical aptasensors according to the Web of Science

Colas et al. [15] further introduced peptide aptamers in 1996, by reporting short peptide structures able to recognize cyclin-dependent kinase 2. Since peptide aptamers are out of the scope of this review, this term will be used here strictly for those bioreceptors based on nucleic acids.

Aptamers are generated through an in vitro combinatorial process called "systematic evolution of ligands by exponential enrichment" (SELEX) [16, 17]. Traditional SELEX is based on repetitive cycles of:

- Incubation of a random pool of oligonucleotide sequences with the target molecule
- Binding of the target molecule to some oligonucleotide
- Partitioning of bound and unbound sequences
- Amplification of target-bound sequences by polymerase chain reaction for DNA sequences or reverse transcription polymerase chain reaction for RNA sequences

In the last 29 years, great effort has been devoted to improve the SELEX process, motivated by the growing interest in aptamer applications. As result, several SELEX variants have been reported, mainly addressed to produce aptamers with high affinity through faster and more efficient methods. However, this topic is beyond the scope of this review, and it has been excellently covered in previous articles [16–18].

Aptamers have excellent characteristics as bioreceptors , some of them similar to or even better than those of antibodies, which make these oligonucleotides excellent affinity bioreceptors for biosensing as well as other applications, such as biomedical imaging, targeted drug delivery, and biomarker discovery. The most relevant properties for electroanalytical applications are as follows:

- Production. Aptamers are screened through an in vitro process and can be further produced at large scale by well-established synthetic chemical methods [19]. Accordingly, this overcomes the use of animals or cell lines that are mandatory for antibody production. It is probably the main advantage over antibodies, because these procedures are less expensive, simpler, and more reproducible. In addition, aptamers can be efficiently produced for targets that often failed in antibody technology, such as low molecular weight inorganic and organic molecules, toxic compounds, and antigens with epitopes similar to those of the protein molecules from the host animal.
- Affinity binding capacity. Aptamers can bind the target compounds via noncovalent multipoint interactions based on the combined contribution of several attractive forces, including hydrogen bonds, electrostatic and hydrophobic interactions, π–π stacking, and van der Waals forces [20]. This multipoint attachment is favored by conformational changes in the aptamer secondary structure, which refold

around a target with complementary molecular shape and chemical structure. Such a binding mechanism allows the formation of highly stable aptamer–target affinity complexes, with dissociation constants ranging from he nanomolar level to the picomolar level, that are comparable to or even better than those from monoclonal antibodies.

- Chemical modification. Proper functionalization of bioreceptors is a key factor to allow further stable, and preferably, oriented immobilization of the electrode surface for biosensor construction. Aptamers are linear oligomers with relatively short size that can be synthesized with a reactive chemical group at the 3′-terminal or 5′-terminal residue. In addition, such a terminal group can be further modified through conventional organic reactions [21, 22]. In contrast, chemical functionalization of antibodies is mainly performed through group-specific but not site-specific reactions, with the risk to transform the amino acid residues involved in the antigen recognition process. Chemical transformation of sugar moieties at the Fc region can avoid this risk but requires multistep derivatization processes that often affect the stability of the modified antibody.
- Thermal and conformational stability. Antibodies are large, multidomain proteins of 150 kDa that experience irreversible unfolding and aggregation on thermal treatment, with the consequent loss of the biorecognition properties [23]. In contrast, aptamers are highly stable single-stranded nucleic acid molecules that can also recover their native, active conformation after thermal denaturation.
- Target-mediated folding equilibrium. Aptamers can fold in different 3D structures that refold on binding to the target molecule [24, 25]. This property allows the establishment of label-free and single-step biosensing strategies, which can be improved through rational design of the aptamer molecule.

Electrochemical aptasensors

Electrochemical aptasensors can be defined as those electroanalytical biosensors that use aptamers as affinity biorecognition elements. Since their discovery, aptamers have attracted considerable attention for electrochemical biosensor construction, resulting in a large number of research articles having been published in this field.

In particular, researchers have been prolific in creating a great variety of aptamer-based biosensing strategies, making it difficult to establish a general classification scheme [26]. In this review we propose a systematic method for aptasensor classification based on the sensing mechanism used and focusing on the approach used to generate the analytical signal. In this sense, most of the electrochemical aptasensors reported in the period covered by this review can be classified into two main groups, which are subdivided according to specific characteristics of each principal category:

1. The redox probe generating the analytical signal is associated with the aptamer sensing molecule.
   - Covalently attached redox probe
   - Noncovalently interacting redox probe
2. The redox probe generating the analytical signal is not associated with the aptamer sensing molecule.
   - Analyte-induced assembly of sensor architecture
   - Analyte-induced disassembly of sensor architecture

In the following sections, representative examples of electrochemical aptasensors recently reported in the literature will be discussed on the basis of this classification scheme.

Redox probe associated with the aptamer sensing molecule

Covalently attached redox probe

This group comprises those devices using a sensing mechanism based on the analyte-induced refolding of aptamers bearing a covalently attached redox probe. This approach was proposed by Plaxco's group [25] as a simple and effective method to construct label-free electrochemical aptasensors.

In general, this sensor design couples a target-induced conformational change in the labeled aptamer molecule with a change in electron transfer ability between a distal-appended redox marker and an electrode surface [27]. Such aptasensors can lead to an increase or a decrease of the electrochemical signal after target recognition based on the initial and final folding structure of the aptamer molecule. Figure 2 presents a structure-switching electrochemical aptasensor with an on to off response.

The main advantages associated with these aptasensor designs can be summarized as follows [10, 27, 28]:

- Fast target-induced conformational changes in the aptamer allow real-time analysis.
- Specific target-mediated structure switching of the bioreceptor significantly reduces the nonspecific electrochemical response in complex media.
- The covalently attached redox probe allows construction of reagentless, label-free aptasensors.
- Simple and often one-pot analysis protocols.

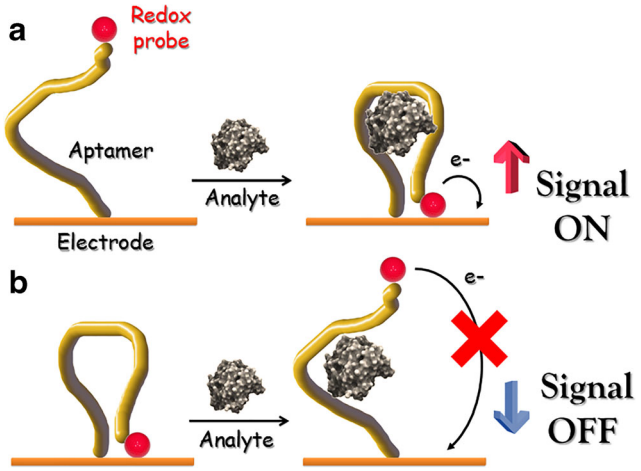

Fig. 2 Structure-switching electrochemical aptasensor with off to on (**a**) and on to off (**b**) responses

- The use of different aptamers labeled with distinct redox probes allows construction of multiplexed sensors for simultaneous multianalyte detection.
- The reversible aptamer folding process could lead to the design of reusable aptasensors and continuous in vivo molecular sensing technologies.

However, this sensing approach has some limitations, such as [27, 29–33]:

- The stability of the aptamer 3D folded structure is a key factor for a proper analytical response, and it is highly affected by several factors, such as temperature, pH, ionic strength, and composition of the medium.
- The optimal aptamer packing density at the electrode surface determines the analytical response of the sensor. Low aptamer packing density will result in low or negligible analytical signal, but high packing density can inhibit target accessibility and aptamer folding because of steric hindrance.
- The sensitivity and limit of detection are limited by the density of the redox probe able to reach the electrode surface, which is determined by the aptamer packing density and the proportion of aptamer molecules properly folded into the active 3D conformation.
- Charges of redox probes can critically affect both the interfacial state of DNA aptamers and electrochemistry of DNA-aptamer-conjugated redox labels, and accordingly, the electroanalytical performance of the aptasensor.
- Aptamers are sensitive to degradation by nucleases.

During recent years, a large number of structure-switching electrochemical aptasensors have been developed for a great variety of analytes. As an example, Chen et al. [34] described the use of a methylene blue-tagged thiolated aptamer as an affinity bioreceptor for the specific detection of interferon-γ (IFNγ) . The aptasensor, assembled on gold microarray electrodes, was able to detect IFNγ in the concentration range from 1 to 500 ng/mL, with a detection limit of 1.3 ng/mL and excellent specificity. A methylene blue-tagged aptamer was also used by Yu and Lai [35] to fabricate a reagentless "signal-on" electrochemical aptasensor for ampicillin that was regenerated and reused, allowing up to three analytical determinations. A similar structure-switching approach was used by Das et al. [36] to determine *Mycobacterium tuberculosis* antigen.

A more complex device was described by Liu et al. [37], who assembled a multiplexed aptasensor for the simultaneous detection of IFNγ and tumor necrosis factor (TNF-α), two important inflammatory cytokines released from T cells. Hairpin aptamers with a thiol group at the 3′ terminus and a redox probe at the 5′ terminus were used as biorecognition elements. To allow multiplexing, two different redox reporters, anthraquinone and methylene blue, were used to label the DNA IFNγ and RNA TNF aptamers, respectively. RNA aptamers are sensitive to RNase degradation, and this problem was addressed by use of phosphorothioates to stabilize the RNA TNF aptamer.

The aptasensor was constructed by randomly attaching the aptamers to a micropatterned gold electrode array, which was further integrated into a microfluidic device as illustrated in Fig. 3. In the presence of the target cytokine, a reduction of the specific redox peak obtained by square-wave voltammetry was observed in a concentration-dependent manner. This multiplexed aptasensor was highly stable and specific, and was able to simultaneously detect IFNγ at a concentration of 6.35 ng/mL and TNF at a concentration of 5.46 ng/mL.

To construct an electrochemical aptasensor for adenosine triphosphate (ATP) with improved analytical properties, Jia et al. [38] used a dual-hairpin DNA structure as a signal amplifier. The rational of this design requires the immobilization of a methylene blue-labeled aptamer on the electrode surface, which was further hybridized with a methylene blue-labeled adjunct probe to form a dual-hairpin structure (Fig. 4). In the absence of the target, the stable dual-hairpin conformation allowed the redox probes to stay close to the electrode surface, generating a strong current signal. However, in the presence of ATP, the aptamer experienced a conformational change leading to separation of the attached redox probe from the electrode surface and forcing the adjunct probe to dissociate from the modified electrode. Such conformational change and architecture disassembly caused a noticeable reduction in the analytical current signal.

Noncovalently interacting redox probe

Redox probes can interact noncovalently with aptamer molecules by electrostatic binding or intercalation, and this property has been used to design electrochemical aptasensors without a redox probe attached.

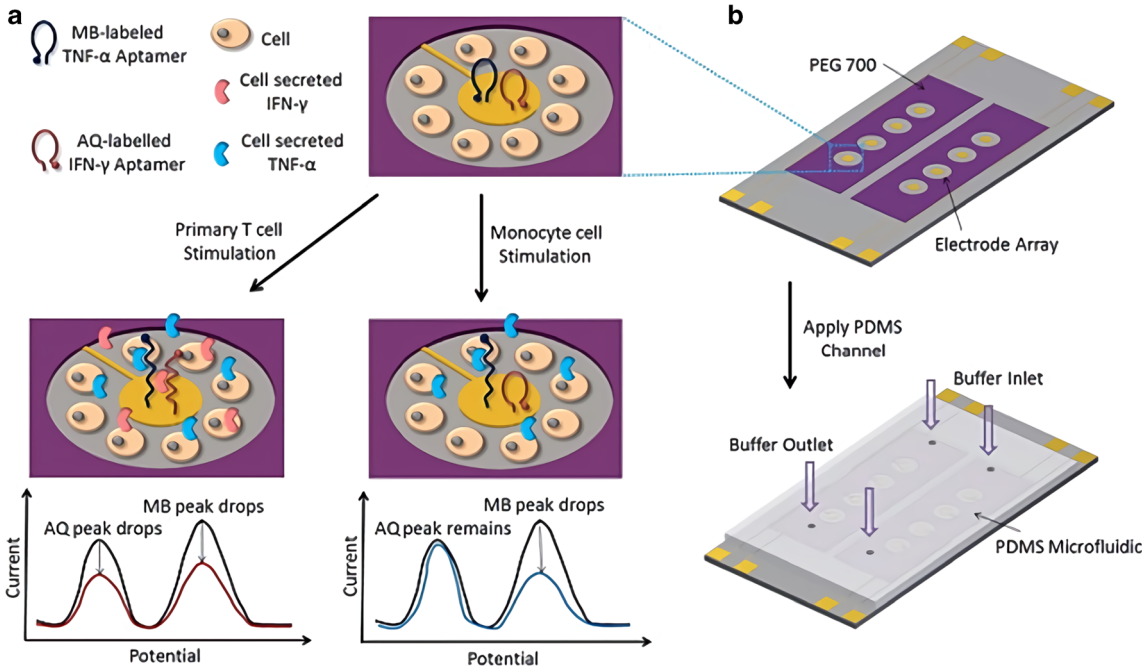

Fig. 3 Microfluidic sensing platform for the aptamer-refolding-based simultaneous detection of interferon-γ (IFNγ) and tumor necrosis factor (TNF) released from T cells. AQ anthraquinone, MB methylene blue, PDMS polydimethylsiloxane. (Reprinted with permission from [37]. Copyright 2014, Elsevier B.V.)

These strategies have substantial precedents from similar and well-established methods used to construct sequence-specific hybridization genosensors based on the preferential interaction of several redox probes, such as cationic metal complexes and organic compounds with double-stranded DNA [39]. As illustrated in Fig. 5, the aptasensor architecture is formed by self-hybridization of the aptamer molecule or hybridization with a complementary strand.

In comparison with the aptasensors described in "Covalently attached redox probe," a high load of intercalated or electrostatically interacting redox probe leads to higher sensitivity and lower detection limits. Such analytical properties

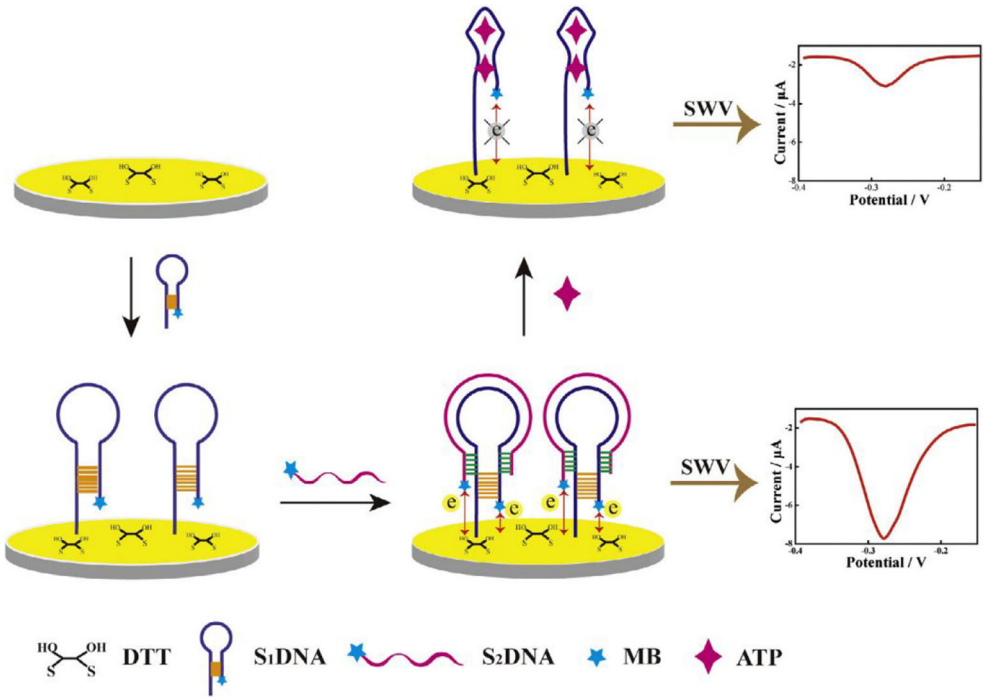

Fig. 4 Detection of adenosine triphosphate (ATP) based on electrochemical signal amplification by a dual-hairpin DNA structure in combination with the insertion approach. DTT dithiothreitol, MB methylene blue, SWV square-wave voltammetry. (Adapted with permission from [38]. Copyright 2015, Elsevier B.V.)

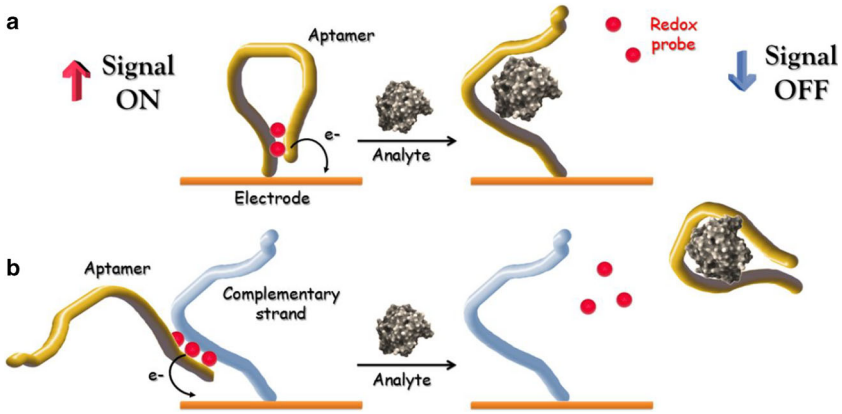

Fig. 5 Electrochemical aptasensors based on the self-hybridization of aptamers (**a**) and hybridization with complementary strand (**b**)

can be also improved by tailored design of sensors with large hybridization regions. However, these aptasensors cannot be operated through a single-step protocol, and the analytical response is obtained by the reduction of the electrochemical signal caused by the release of the redox probe from the electrode surface due to the target-induced dissociation of aptamer hybridization.

As an example of aptasensors based on this self-hybridization of aptamer molecules, Tabrizi et al. [40] reported an electrochemical method for aptamer-based determination of insulin at femtomolar concentrations by using methylene blue as a redox probe. This sensor was assembled on screen-printed electrodes coated with ordered mesoporous carbon chemically modified with 1,3,6,8-pyrenetetrasulfonate to further attach the amino-terminated aptamer via reactive sulfonyl chloride groups. Finally, the redox probe methylene blue was intercalated into the aptamer. The sensing approach relies on the release of the redox probe from the aptamer after binding to insulin, leading to a decreased differential pulse voltammetry signal. This aptasensor was able to quantify insulin with high selectivity and sensitivity in the 1.0 fM to 10.0 pM concentration range, with a limit of detection of 0.18 fM. A similar sensing approach was recently used by Yu et al. [41] to detect bisphenol A, by intercalating methylene blue into a specific aptamer with a hairpin structure previously immobilized on the electrode surface.

Following a similar strategy, an ultrasensitive aptasensor for the anti-inflammatory drug ibuprofen was developed by coating a glassy carbon electrodes with a multiwalled carbon nanotube/ionic liquid/chitosan nanocomposite and further covalent immobilization of a specific aptamer [42]. Methylene blue was then intercalated into the aptamer molecule as an electrochemical redox marker. The decrease in the differential pulse voltammetry signal of the redox reporter on incubation with the target analyte was directly proportional to the drug concentration in the range from 70 pM to 6 μM with a low detection limit of 20 pM.

Target-induced complementary strand displacement strategies have attracted less interest because of disadvantages associated with the assembly of such highly complex sensor architectures. In this field, Wei and Zhang [43] reported the use of gold electrodes modified with a specific aptamer for ochratoxin A to construct a voltamperometric aptasensor for this mycotoxin. To amplify the analytical signal, the aptamer was hybridized with a complementary DNA strand loaded on carbon aerogels, and methylene blue was then intercalated as a redox probe. This approach allowed detection of ochratoxin A in the range from 0.10 to 10 ng/mL with a limit of detection of 0.1 pg/mL.

A simpler sensor architecture was recently used by Raouafi et al. [44] to construct an electrochemical aptasensor for prostate-specific antigen (PSA) by using carbon screen-printed electrodes coated with carboxylated graphene as support for the covalent immobilization of the aptamer molecules. The aptasensor assembly involved the further hybridization of a partially complementary DNA strand and intercalation of methylene blue into the double-stranded DNA sequences. The aptasensor allowed specific and selective detection of the cancer antigen from 1 pg/mL to 100 ng/mL with a low detection limit of 64 fg/mL.

Redox probe not associated with the aptamer sensing molecule

This category comprises a huge group of aptasensors for which the analytical signal is generated by the diffusion to the electrode surface of a redox probe from the bulk solution or generated in situ by an enzymatic or similar signaling approach. The main advantages of such methods can be summarized as follows:

- Possibility to assemble a large variety of sensor architectures that can involve the use of complementary aptamers, antibodies, lectins, and other bioreceptors, and labeling with enzymes, nanomaterials, etc.
- Sensing mechanism could involve assembly or disassembly of the sensor architecture.

- Allow the design of original signal amplification strategies
- Can be adapted to aptasensors for label-free and one-pot analysis
- Excellent analytical properties in terms of linear range of response, sensitivity, and limit of detection
- Can be constructed to detect any type of analyte

In contrast, complex aptasensor architectures and sensing approaches can affect reproducibility and could require multistep measuring protocols. In addition, the sensitivity of some label-free aptasensor designs will be determined mainly by the electroanalytical technique employed.

In the following sections, recent examples of aptasensors with a redox probe not associated with the aptamer sensing molecule are discussed on the basis of the sensing approach used to detect proteins, cells and low molecular weight compounds.

Analyte-induced assembly of sensing architecture

In this subcategory, the simple sensing approach is based on the formation of an aptamer–analyte affinity complex at the aptamer-modified electrode surface and the associated changes in the electrochemical behavior of the electrode. This method was applied to construct several label-free aptasensors for proteins. As an example, an impedimetric aptasensor for PSA was constructed by Karimipour et al. [45] by using glassy carbon electrodes modified with TiO_2(200)-reduced graphene oxide nanohybrid as support for the aptamer immobilization. In other studies, Arya et al. [46] reported the assembly of a capacitive aptasensor for human epidermal growth factor receptor 2 based on aptamer-functionalized interdigitated gold electrodes, and Grabowska et al. [47] constructed aptamer-based electrochemical sensors for brain natriuretic peptide and cardiac troponin I by using gold screen-printed electrodes coated with polyethyleneimine/reduced graphene oxide films as the sensing surface.

Fenzl et al. [48] reported the design of a label-free voltamperometric biosensor for the coagulation factor thrombin that used laser-scribed graphene (LSG) electrodes anchored with 1-pyrenebutyric acid as support for the covalent immobilization of the specific antithrombin aptamer. Figure 6 presents the detection mechanism for this aptasensor, which is based on the specific binding of thrombin to the immobilized aptamers and the resulting hindrance of diffusion of the redox marker thus causing a decrease in the peak currents obtained from voltammetric measurements. This aptasensor showed extremely low detection limits of 1 pM in buffer and 5 pM in serum.

In other work, a portable aptamer-based field-effect transistor biosensor for *Plasmodium falciparum* glutamate dehydrogenase was recently constructed by immobilization of a DNA aptamer on interdigitated gold microelectrodes (Fig. 7) [49]. This aptasensor, based on the analyte-induced assembly of an affinity adduct at the electrode surface, exhibited high selectivity and sensitive response in a broad dynamic range from 100 fM to 10 nM with limits of detection of 16.7 pM in spiked buffer and 48.6 pM in serum sample,. These analytical characteristics allowed this device to be proposed for diagnosis of symptomatic and asymptomatic malaria.

Argoubi et al. [50] described a label-free electrochemical aptasensor for PSA based on gold electrodes coated with a thin film of mesoporous silica (Fig. 8). The sensing approach also relies on hindering the diffusion of the redox probe to the electrode surface, but here the diffusion mechanism involves passage through the nanochannels of the mesoporous film covalently functionalized with the specific DNA aptamer. This aptasensor was highly sensitive and specific for PSA in the range from 1 to 300 ng/mL, with a low detection limit of 280 pg/mL.

Jolly et al. [51] proposed a sensor design that combines the biomolecular recognition capacity of aptamers with molecularly imprinted polymer technology. Gold electrodes were functionalized with a thiolated DNA aptamer and further incubated with PSA to produce the affinity complex (Fig. 9). Controlled electropolymerization of dopamine was further performed to entrap the complex, hold the aptamer in its binding conformation, and localize the PSA-binding sites at the sensor interface.

Removal of PSA allowed exposure of the hybrid aptamer–molecularly imprinted polymer binding sites on the electrode surface, which displayed greater recognition than the aptamer alone. This aptasensor showed high sensitivity and specificity toward PSA, with linear range of response from 100 pg/mL to 100 ng/mL and a limit of detection of 1 pg/mL.

Wang et al. [52] constructed a sandwich-type aptasensor for thrombin by using glassy carbon electrodes coated with WSe_2 nanosheets and gold nanoparticles as support for the stable immobilization of the capture aptamer (Fig. 10). After biorecognition of the protein analyte, a signal nanoprobe based on gold nanoparticles bifunctionalized with a biotinylated DNA strand and the thrombin aptamer was assembled, allowing recording of the analytical signal after incubation with a streptavidin–alkaline phosphatase conjugate. This aptasensor was also empowered by use of an electrochemical–chemical–chemical redox cycling and enzyme signal enhancement strategy, allowing the achievement of excellent analytical properties, such as a linear range of 0–1 ng/mL and a low detection limit of 190 fg/mL for thrombin. In other work, Kim et al. [53] used a cognate pair of aptamers and screen-printed gold electrodes modified with coccolith and electrodeposited gold nanoparticles to assemble a sandwich-type electrochemical biosensor for vaspin, a type 2 diabetes biomarker.

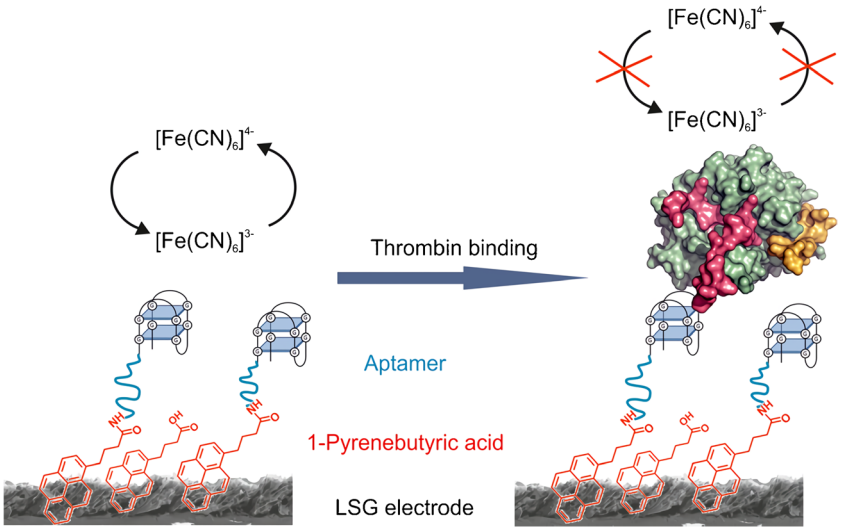

Fig. 6 Sensing mechanism for thrombin detection using aptamer-modified laser-scribed graphene (LSG) electrodes. (Adapted with permission from [48]. Copyright 2017 American Chemical Society)

When the secondary aptamer is not available because the target molecular sequence is recognized and covered by the primary aptamer strand, an antibody and aptamer pair can be used to construct sandwich-type biosensing schemes [54]. This approach was used by Shui et al. [55] to construct an antibody–aptamer sandwich assay for tau-381 protein, a potential biomarker for Alzheimer's disease. The capture antibody was covalently attached to 3-mercaptopropionic acid-modified gold electrodes and the signal amplification element was prepared with cysteamine-stabilized gold nanoparticles coated with a specific aptamer. Differential pulse voltammetry measurements allowed detection down to 0.42 pM for tau-381, and the device was validated in human serum from patients with Alzheimer's disease.

Lee et al. [56] recently used an aptamer–antibody sandwich scheme to determine tyrosine kinase 7 by differential pulse voltammetry as the electroanalytical technique. This device was constructed by covalent immobilization of the capture aptamer on gold-nanoparticle-modified screen-printed carbon electrodes, and the sensing approach involved binding of an alkaline-phosphatase-conjugated antibody to the aptamer–tyrosine kinase 7 affinity complex.

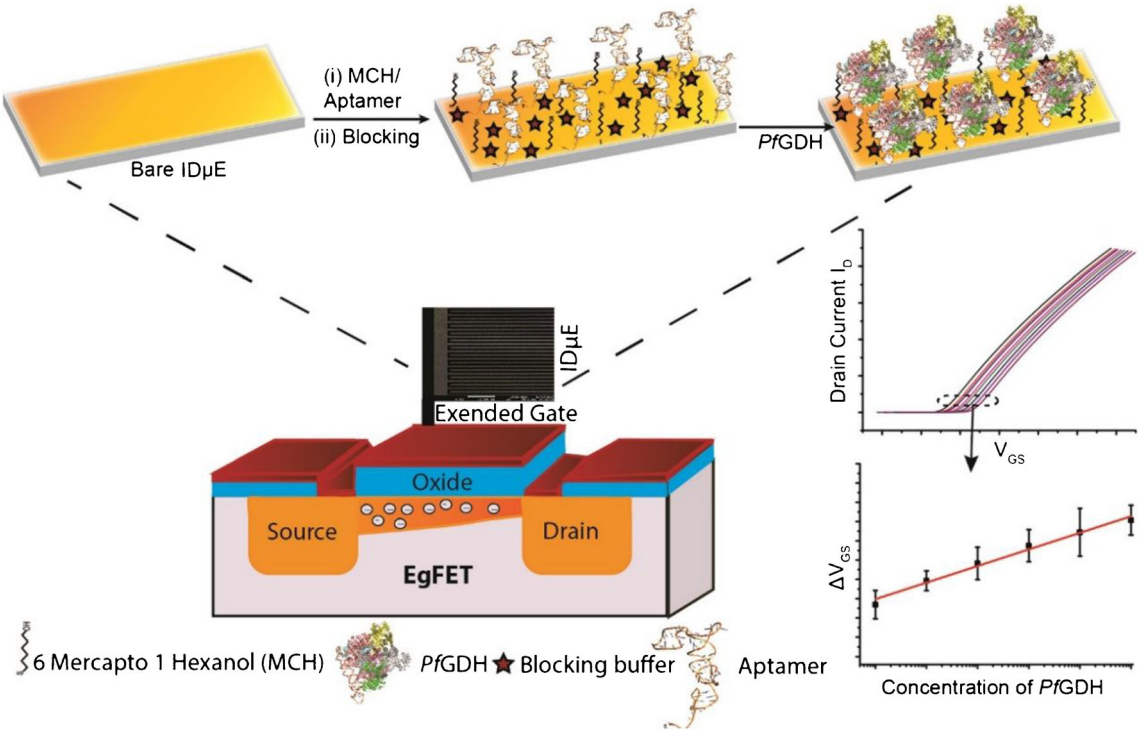

Fig. 7 Fabrication scheme for the extended-gate field-effect transistor (EgFET) aptasensor for the detection of *Plasmodium falciparum* glutamate dehydrogenase (*Pf*GDH) in blood serum. IDμE interdigitated microelectrode. (Adapted with permission from [49]. Copyright 2018 Elsevier B.V.)

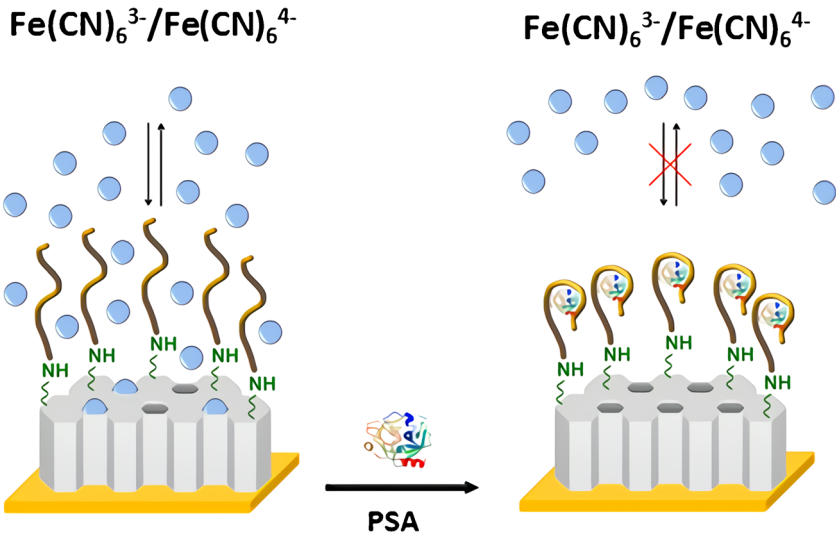

Fig. 8 Sensing mechanism for prostate-specific antigen (PSA) detection using gold electrodes coated with a thin film of mesoporous silica thin and functionalized with anti-PSA aptamer. (Adapted with permission from [50]. Copyright 2017 Elsevier B.V.)

In other work, Chung et al. [57] developed a magnetic-force-assisted electrochemical aptamer–antibody sandwich assay to detect thrombin in serum samples. The capture interface was constructed by covalent immobilization of a thrombin-specific aptamer on screen-printed carbon electrodes coated with poly(2,2′:5′,5″-terthiophene-3′-*p*-benzoic acid). On the other hand, magnetic nanoparticles coated with starch and streptavidin and further conjugated with biotinylated thrombin antibodies and toluidine blue O were used as a detection element.

Thrombin was determined by the cathodic currents of toluidine blue O after assembly of the sandwich-type adduct. The main novelty of this research is that removal of unbound magnetic bioconjugates from the sensor surface is controlled

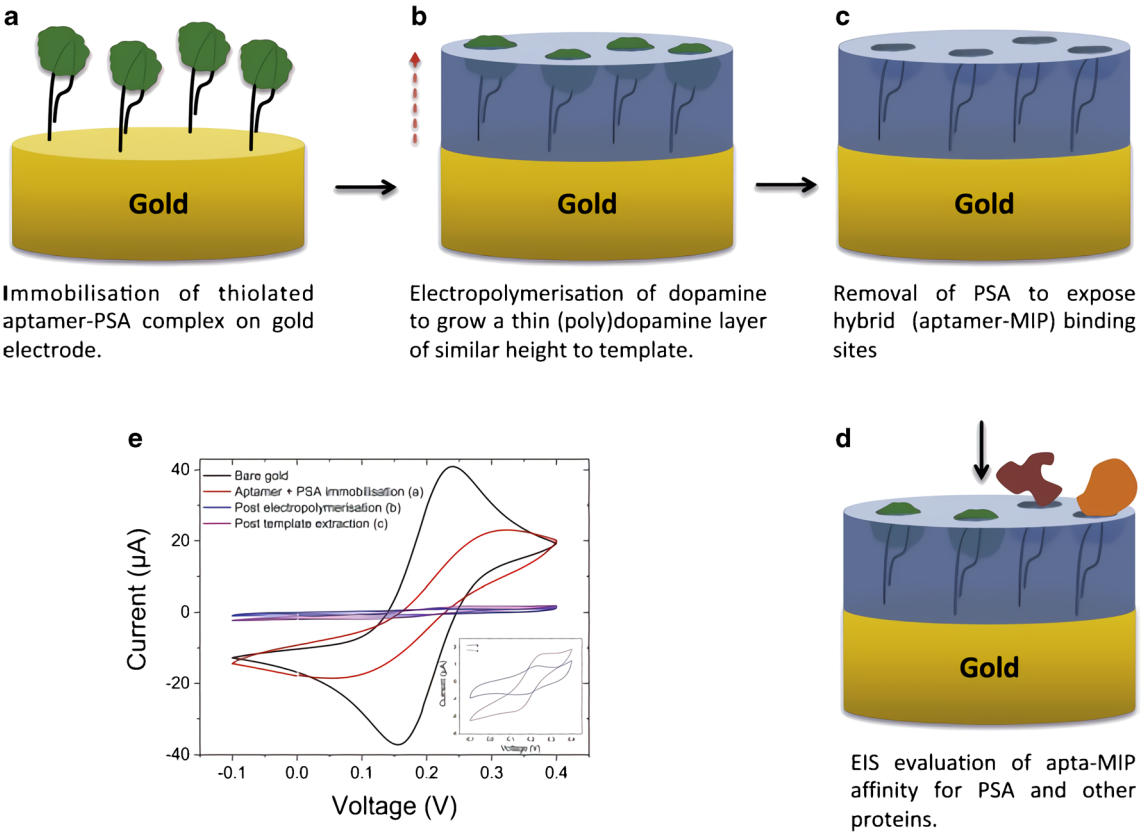

Fig. 9 **a**–**d** Steps involved in the construction of the aptamer–molecularly imprinted polymer (MIP) biosensor for prostate-specific antigen (PSA) detection, and e cyclic voltammetry monitoring of the fabrication process. EIS electrochemical impedance spectroscopy. (Adapted from [51]. Copyright 2015 Elsevier B.V.)

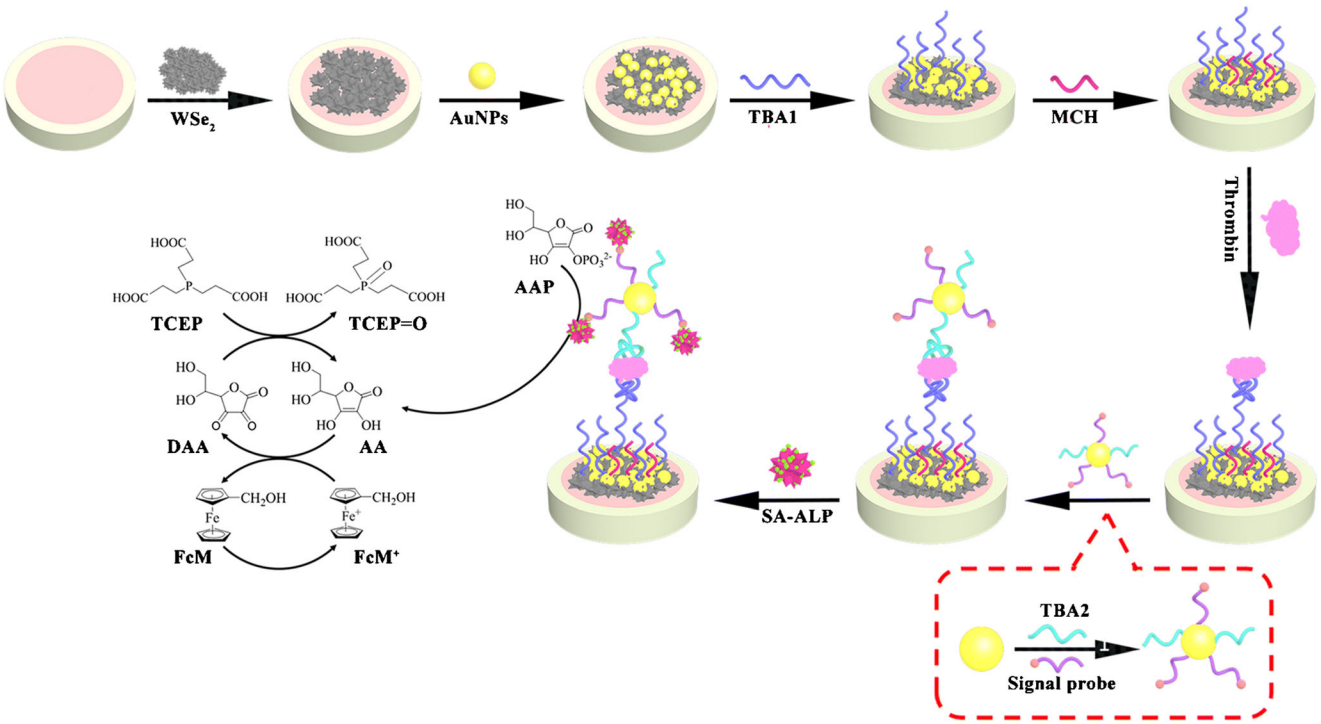

Fig. 10 Assembly and sensing mechanism for the sandwich-type aptasensor for thombin detection as described by Wang et al. [52]. The signal probe is a biotinylated DNA strand. AA ascorbic acid, AAP ascorbic acid 2-phosphate, AuNPs gold nanoparticles, DAA dehydroascorbic acid, FcM ferrocenemethanol, MCH 6-mercapto-1-hexanol, SA-ALP streptavidin–alkaline phosphatase conjugate, TBA1 capture aptamer, TBA2 detection aptamer, TCEP tris(2-carboxyethyl)phosphine, TCEP=O tris(2-carboxyethyl)phosphine oxide. (Adapted with permission from [52]. Copyright 2018, Springer-Verlag GmbH, Austria, part of Springer Nature)

by a magnetic field without washing steps, as illustrated in Fig. 11.

Wang et al. [58] proposed a sensing scheme that takes advantage of the sugar biorecognition properties and multibinding capacity of concanavalin A to design an aptamer–lectin sandwich assay for carcinoembryonic antigen (CEA) detection. The rational of this assay, as illustrated in Fig. 12, was based on the use of an specific anti-CEA aptamer

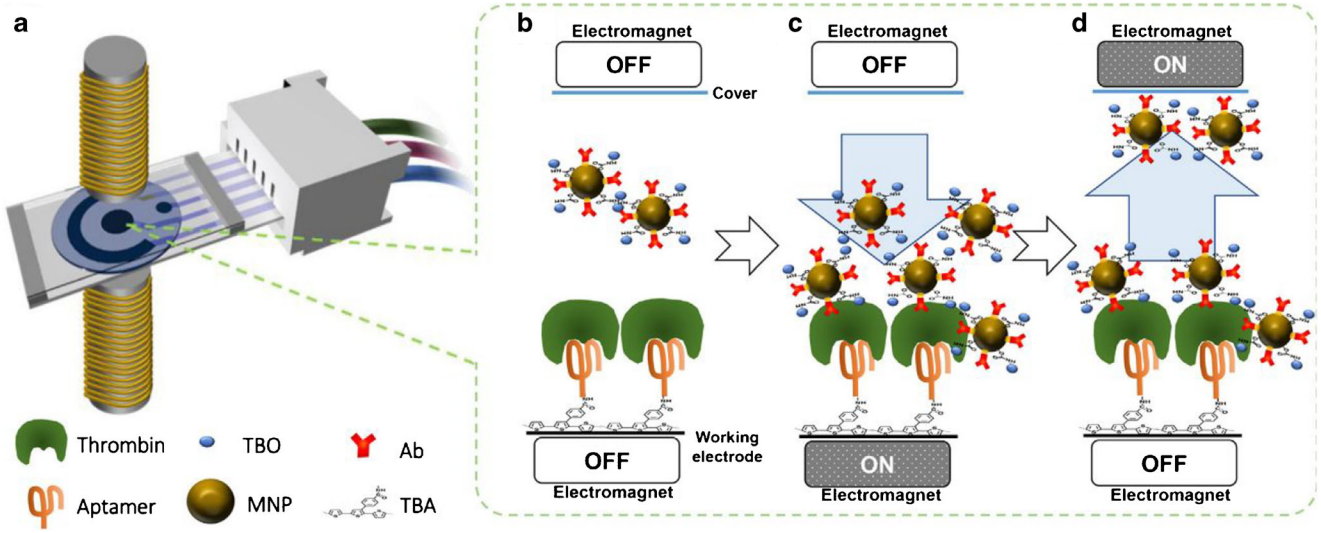

Fig. 11 Device and sensing approach for thrombin detection using a magnetic-force-assisted electrochemical aptamer–antibody sandwich assay. The device is composed of an electrochemical sensor, a pair of electromagnets, and a sample chamber (A). The sensing assay includes the sample loading step to mix the sample solution and the magnetic nanoparticle bioconjugates (B), the reaction step to form sandwich complexes on the electrode surface (C), and the removal step to remove unbound magnetic nanoparticle bioconjugates from the electrode surface (D). Ab antibody, MNP magnetic nanoparticle, TBA 2,2′:5′,5″-terthiophene-3′-*p*-benzoic acid, TBO toluidine blue O. (Adapted with permission from [57]. Copyright 2018, Elsevier B.V.)

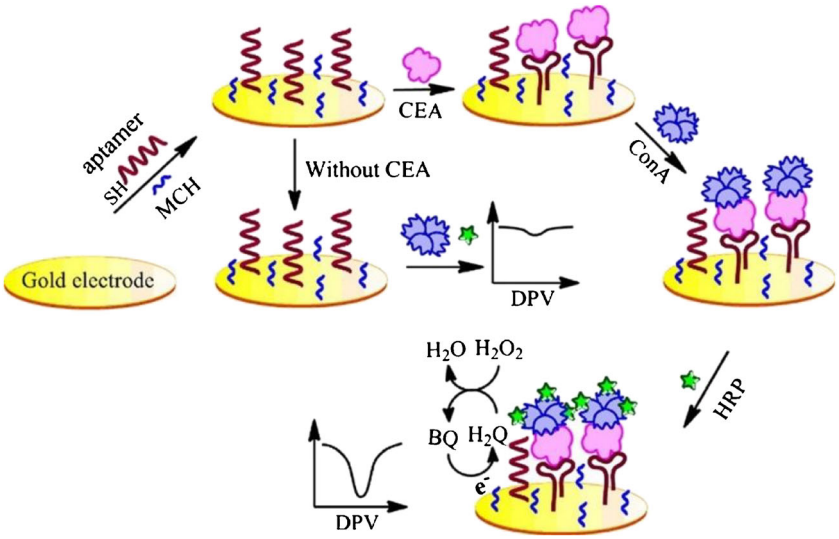

Fig. 12 Assembly and sensing mechanism for carcinoembryonic antigen (CEA) determination by use of the aptamer–lectin sandwich assay. BQ benzoquinone, ConA concanavalin A, DPV differential pulse voltammetry, H_2Q hydroquinone, HRP horseradish peroxidase, MCH 6-mercapto-1-hexanoic acid. (Adapted with permission from [58]. Copyright 2017, Elsevier B.V.)

as a capture element on the electrode surface and concanavalin A as a secondary biorecognition element also able to bind horseradish peroxidase for signal production and amplification. It should be highlighted that horseradish peroxidase was used as a noncovalent label for concanavalin A, although the authors claimed a label-free scheme. This electrochemical aptasensor was used to quantify CEA from 5 to 40 ng/mL with a detection limit of 3.4 ng/mL.

In recent work, we described a novel biosensing strategy for CEA by using aptamer-functionalized Janus nanoparticles as an integrated electrochemical biorecognition-signaling element [59]. Engineered gold–silica Janus nanoparticles were functionalized with horseradish peroxidase on the silica surface to act as a signaling element and a biotin-labeled anti-CEA DNA hairpin aptamer at the gold face to assemble the biorecognition element. As illustrated in Fig. 13, the sensing mechanism relies on the unfolding of the DNA hairpin structure and unmasking of the biotin residue on recognition of the target biomarker, allowing association with avidin-modified Fe_3O_4–SiO_2 core–shell supermagnetic nanoparticles (NanoCaptors®). Further magnetic deposition on carbon screen-printed electrodes allowed the amperometric detection of CEA in the range from 1 to 5000 ng/mL with a limit of detection of 210 pg/mL.

Jiang et al. [60] reported a more complex biosensor design involving target-induced assembly of sensing architectures to detect thrombin. As illustrated in Fig. 14, this sensing approach relies on the initial binding of thrombin to the aptamer-containing hairpin probes immobilized on the electrode surface, triggering the catalytic assembly of two other hairpins to form many G-quadruplex Y-junction DNA structures in situ. The as-formed G-quadruplex ensembles further associate with hemin to form G-quadruplex–hemin complexes, allowing the electrochemical detection of the attached porphyrin. This aptasensor showed excellent analytical properties, with a linear range of response of 0.01–1.0 nM and a detection limit of 6.0 pM for thrombin.

The same research group also developed a dual amplified signal enhancement strategy based on the integration of catalytic hairpin assembly and terminal deoxynucleotidyl

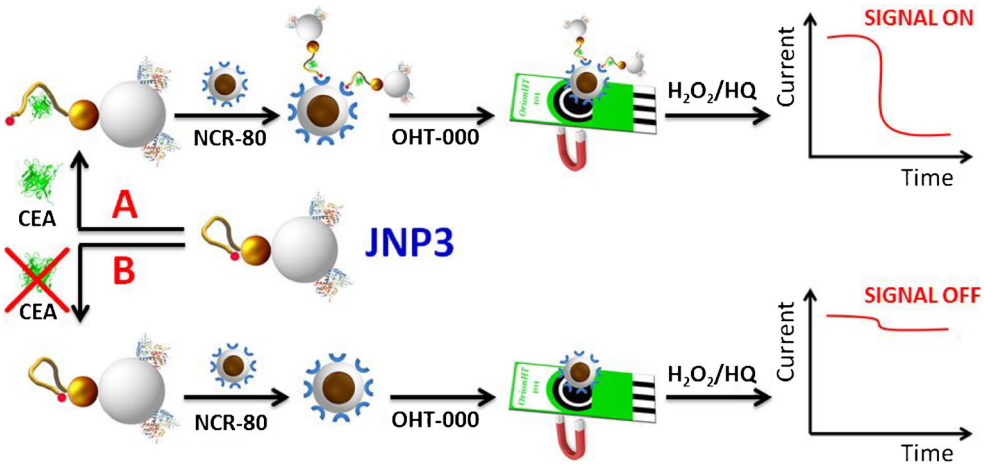

Fig. 13 Janus nanoparticle (JNP)-based biosensing strategy for carcinoembryonic antigen (CEA) detection using avidin-modified magnetic NanoCaptors (NCR-80) and screen-printed carbon electrodes (OHT-000). HQ hydroquinone. (Adapted with permission from [59]. Copyright 2019, Elsevier B.V.)

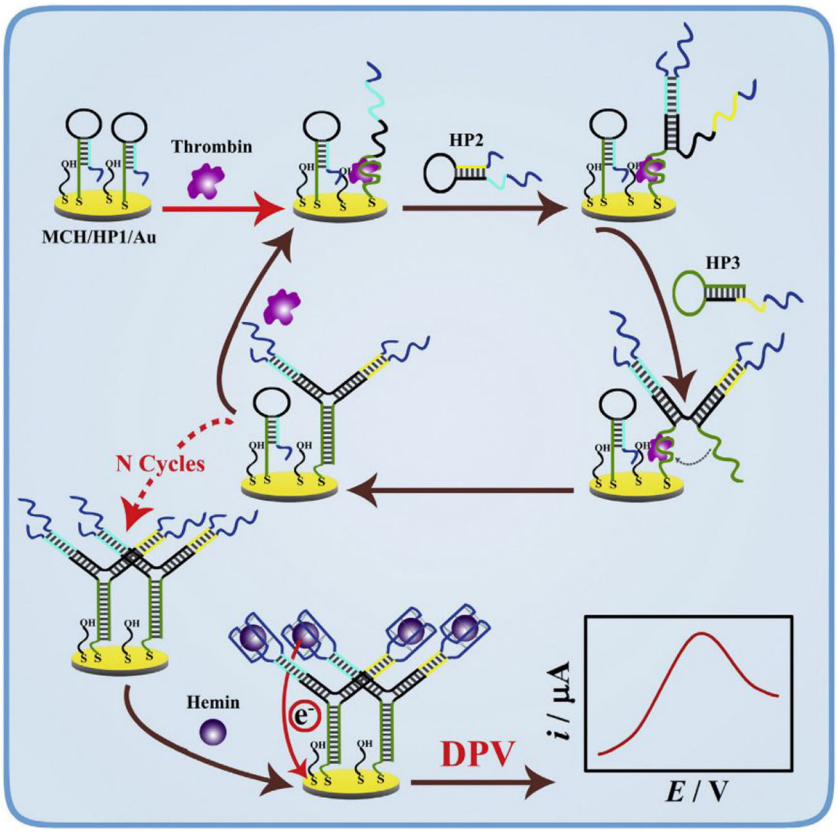

Fig. 14 Working principle for target-induced catalytic hairpin (HP) assembly formation of G-quadruplex–hemin Y-junction structures for amplified detection of thrombin. DPV differential pulse voltammetry, MCH 6-mercapto-1-hexanoic acid. (Adapted with permission from [60]. Copyright 2017, Elsevier B.V.)

transferase-mediated in situ DNA polymerization [61]. The rational of this approach was based on the target-induced unfolding of the hairpin structure of the capture aptamer, leading to the subsequent terminal deoxynucleotidyl transferase-catalyzed elongation of the signal probe and formation of G-quadruplex sequence replicates with the presence of deoxyguanosine triphosphate and deoxyATP (Fig. 15). Finally, the G-quadruplex sequences bind hemin and generate an amplified current response, allowing thrombin detection in the range from 0.5 pM to 10.0 nM and a detection limit of 0.12 pM.

Increased attention have been paid to the construction of aptamer-based biosensors for human cells and pathogenic microorganisms. Zhang et al. [62] proposed a nonconventional electrochemical aptasensor concept by assembling the sensing device on an ion-selective-electrode. Porous graphene oxide, prepared by hydrothermal cross-linking of graphene oxide with thiourea, was used as the coating nanomaterial for an iodide-selective electrode, and a specific aptamer-targeted A549 cell was covalently anchored on the nanostructured surface. This device showed a cell and ion double-sensing feature, with a resolution in blood of ten cells per milliliter.

Proper labeling of aptamers with enzymes, nanomaterials, and/or redox probes allows their use as detection and signal amplification elements in sandwich-type electrochemical biosensors [63]. This strategy was used by Chen et al. [64] to construct an electroanalytical device for dynamic cell surface *N*-glycan determination in CCRF-CEM cells (human acute lymphoblastic leukemia) [64]. CCRF-CEM cells were used as a model because of the association of fatal immune-deficiency-associated diseases with the abnormal glycan expression on their cell surface [65].

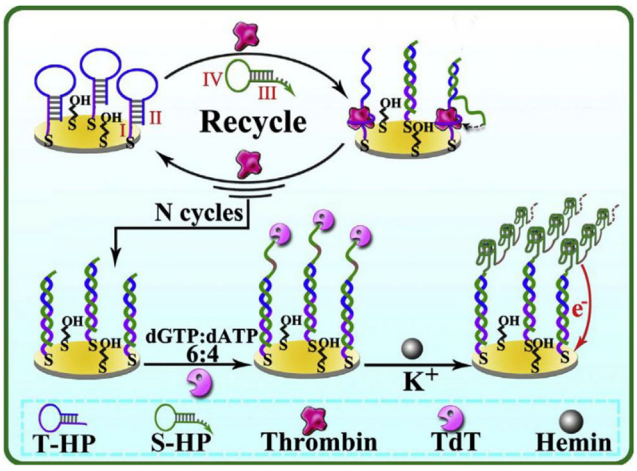

Fig. 15 Label-free and dual amplified electronic thrombin assay protocol based on target-triggered catalytic hairpin assembly and terminal deoxynucleotidyl transferase (TdT)-catalyzed DNA polymerization. dATP deoxyadenosine triphosphate, dGTP deoxyguanosine triphosphate, S-HP signal hairpin probe, T-HP thiol-modified hairpin immobilization probe. (Adapted with permission from [61]. Copyright 2016, Elsevier B.V.)

The transduction interface for this biosensor was assembled by the layering of reduced graphene oxide, poly(amidoamine) generation 3.0 dendrimer, and concanavalin A on a glassy carbon electrode. On the other hand, an original detection nanoprobe was prepared by attachment of horseradish peroxidase and a specific aptamer for CCRF-CEM cells to gold nanoparticles. This biosensor had a low detection limit of ten cells per milliliter, had high selectivity, and was also successfully applied for *N*-glycan expression inhibitor screening. Despite the analytical properties showed by this device, the use in real human samples would require previous isolation of the target cells through tedious procedures because of ability of concanavalin A to recognize a large variety of cells expressing surface glycoproteins with terminal α-D-mannosyl and α-D-glucosyl groups [66]. The use of specific antibodies or aptamer as a capturing element on the electrode surface would increase the practical opportunities for this biosensor.

Another example of an electrochemical aptasensor with a sandwich-type architecture was reported for the pathogenic *Staphylococcus aureus*, by use of aptamer-functionalized magnetic beads as the capture probe and aptamer-coated silver nanoparticles as the signal element [67]. Anodic stripping voltammetry was used to detect the attached silver nanoparticles. The aptasensor showed an extended dynamic range from 10 to 10^6 colony-forming units (CFU) per milliliter and a low detection limit of 1.0 CFU/mL. Surprisingly, the authors used the term "immunosensor" to classify this device although the sensing scheme is not based on an antigen–antibody biorecognition process.

Recently, Brosel-Oliu et al. [68] used a 3D interdigitated electrode array (3D-IDEA) to construct a highly sensitive impedimetric aptasensor for the detection and quantification of pathogenic *Escherichia coli* O157:H7. As illustrated in Fig. 16, $TaSi_2$ and SiO_2 were used as highly conductive and highly isolating materials, respectively, for 3D-IDEA preparation. Proper functionalization of the silicon dioxide layers with 3-mercaptopropyltrimethoxysilane allowed further immobilization of the thiol-functionalized aptamer molecules through the formation of disulfide linkages. On recognition of the *E. coli* strain by the bioreceptor at the 3D-IDEA surface, an electrical charge redistribution is provoked, leading to changes in the surface conductivity. This aptasensor showed a linear response toward the logarithm of bacterial concentration in the range from 10 to 10^5 CFU/mL, with a limit of detection of 10^2 CFU/mL. This device was highly selective for *E. coli* O157:H7, not showing an impedimetric response toward other bacterial strains such as *E. coli* K12, *Salmonella enterica* subsp. *enterica* serovar Typhimurium, and *S. aureus*.

One of the main advantage of using aptamers as affinity recognition elements is their ability to recognize low molecular weight inorganic and organic compounds. Li et al. [69] recently reported the construction of a sensitive aptasensor for ATP based on glassy carbon electrodes modified with a graphene oxide/poly(3,4-ethylenedioxythiophene) composite film and the zwitterionic peptide EKEKEKE. This nanostructured electrode surface was used to immobilize a specific aptamer, allowing simple and one-pot detection of ATP at 0.18 V (vs the saturated calomel electrode) with hexacyanoferrate as the electrochemical probe.

An alternative assembly mechanism is used in aptasensors based on competitive assays, in which the analyte is initially attached at the electrode surface instead of the aptamer molecule. Eissa et al. [70] used this approach to construct a label-free competitive aptamer-based impedimetric biosensor for brevetoxin 2 that was able to detect the marine neurotoxin at a level as low as 106 pg/mL.

Stable and oriented immobilization of bioreceptors on the electrode surface is a key factor to improve the analytical performance of affinity biosensors. Kazane et al. [71] described the stable immobilization of a bisphenol A-specific aptamer, functionalized with a pentahistidine peptide, on electropolymerized poly(pyrrole–nitrilotriacetic) acid film through the formation of chelating linkages. Although not proved by the authors, this noncovalent and reversible immobilization approach offers the advantage of reuse of the electrode for repeated immobilization of histidine-tagged bioreceptors. The aptamer-modified electrode was used to construct a label-free impedimetric aptasensor able to detect bisphenol A from 10^{-11} to 10^{-6} M with excellent specificity.

Analyte-induced disassembly of sensing architecture

In our opinion, the most significant achievements in this subcategory are based on the use of graphene and graphene derivatives as support for the simple construction of electrochemical aptasensors. Graphene and its derivatives can effectively adsorb DNA molecules on their planar surface [72–74], and this property allows construction of DNA-aptamer-based electrochemical biosensors. The most common strategy to prepare such aptasensors involves the adsorption of a DNA aptamer on the electrode surface previously modified with a graphene derivative. In such cases, the sensing approach relies on the analyte-induced desorption of the aptamer molecule and the concomitant changes in the electrochemical behavior of the sensing interface, allowing one-pot and label-free measurements.

This approach was used by Ahour and Ahsani [75] to construct a sensitive label-free aptasensor for thrombin by use of a graphene oxide-modified pencil graphite electrode as a transducer. The main advantages of this device are the simple sensor architecture, based on the adsorption of the aptamer molecule on the nanostructured electrode surface, and the electrochemical sensing approach based on the quantitative determination of the guanine oxidation process. A similar target-induced disassembly strategy was used by Eissa and Zourob

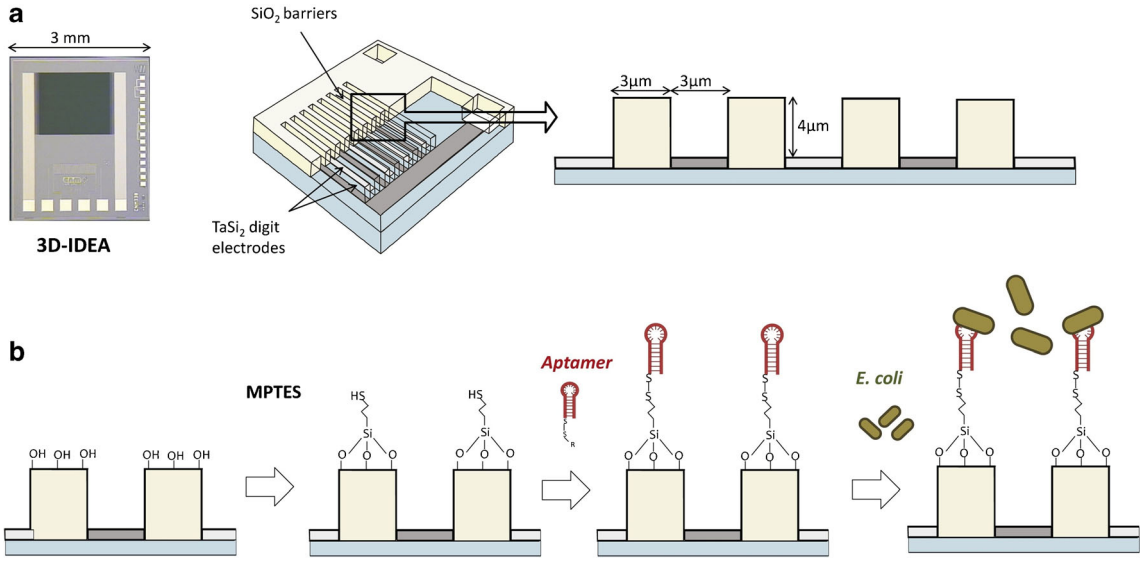

Fig. 16 Three-dimensional interdigitated electrode array (3D-IDEA) sensor surface image and its schematic representation (**a**), and different biofunctionalization steps involved in the assembly of the impedimetric aptasensor for pathogenic *Escherichia coli* O157:H7 (**b**). MPTES 3-mercaptopropyltrimethoxysilane. (Adapted with permission from [68]. Copyright 2018, Elsevier B.V.)

[76] to design a voltamperometric aptasensor for the food allergen β-lactoglobulin using graphene-modified screen printed carbon electrodes and a new aptamer selected by these researchers. Yu et al. [77] also used a target-induced detaching of DNA aptamer from an electrochemically reduced graphene oxide electrode to detect lead ion at ultratrace levels.

To gain insight into the effect of the nanomaterial composition on the physical adsorption capacity of aptamers and, accordingly, their potential use in target-induced detachment electrochemical aptasensors, Aceta and Del Valle [78] prepared graphene oxide samples with different oxidation degrees. These derivatives were used as coating materials for graphite–epoxy composite electrodes and were compared with those coated with electrochemically reduced graphene oxide by use of a thrombin aptamer as a model. Impedimetric experiments demonstrated that highly electrochemically reduced graphene oxide cannot be used in the proposed aptasensing scheme, because the absence of active points in the nanomaterial surface made impossible the aptamer immobilization by physical adsorption. In contrast, oxidized graphene was confirmed as a suitable support for DNA aptamer adsorption, allowing sensitive and label-free detection of thrombin.

Conversely, Jain et al. [79] reported an aptasensor for *Plasmodium* lactate dehydrogenases based on DNA aptamers physically adsorbed on graphene oxide-coated electrodes, but using a different sensing mechanism. In this case, the electrochemical signals were related to the formation of the affinity complex at the electrode surface without detachment of the aptamer molecule. This unexpected result was ascribed to the low degree of coverage of the electrode with the graphene derivative, allowing great immobilization of the aptamer on the raw electrode material instead of on the graphene oxide sheets.

DNA molecules are excellent building blocks for constructing stable self-assembled 3D nanostructures able to experience dynamic stimulus-responsive reconfiguration [80]. A common approach for switching DNA assemblies is a complementary-strand-mediated displacement, but the use of specific DNA aptamers in such 3D structures opens the possibility to triggering their reconfiguration by a broad spectrum of target molecules, including proteins, cells, microorganisms, and chemical compounds. This strategy has been largely used to construct electrochemical aptasensors based on DNA 3D arrangements.

As an example, Taghdisi et al. [81] reported the construction of a Y-shaped DNA architecture comprising two aptamers and a complementary strand, which was assembled on screen-printed gold electrodes for the ultrasensitive detection of myoglobin. As shown in Fig. 17, the Y-shaped ensemble is resistant to exonuclease I treatment, hindering the diffusion of the redox probe to the electrode surface. In the presence of myoglobin, the aptamer–target complexes leave the sensor interface, disassembling the Y-shaped structure and promoting the exonuclease I-mediated degradation of the DNA strand remaining at the electrode surface. Accordingly, a strong electrochemical signal is recorded because of the free diffusion of the redox probe to the transducer, allowing detection of myoglobin concentrations as low as 27 pM.

Disassembly of DNA architectures on aptasensor surfaces has also been applied to quantify low molecular weight analytes. As an example, Wang et al. [82] prepared a voltamperometric aptasensor for ATP by binding a methylene blue-labeled aptamer to a complementary DNA strand attached to gold nanowire electrodes. In the presence of ATP, the labeled aptamer dissociated from the duplex DNA because

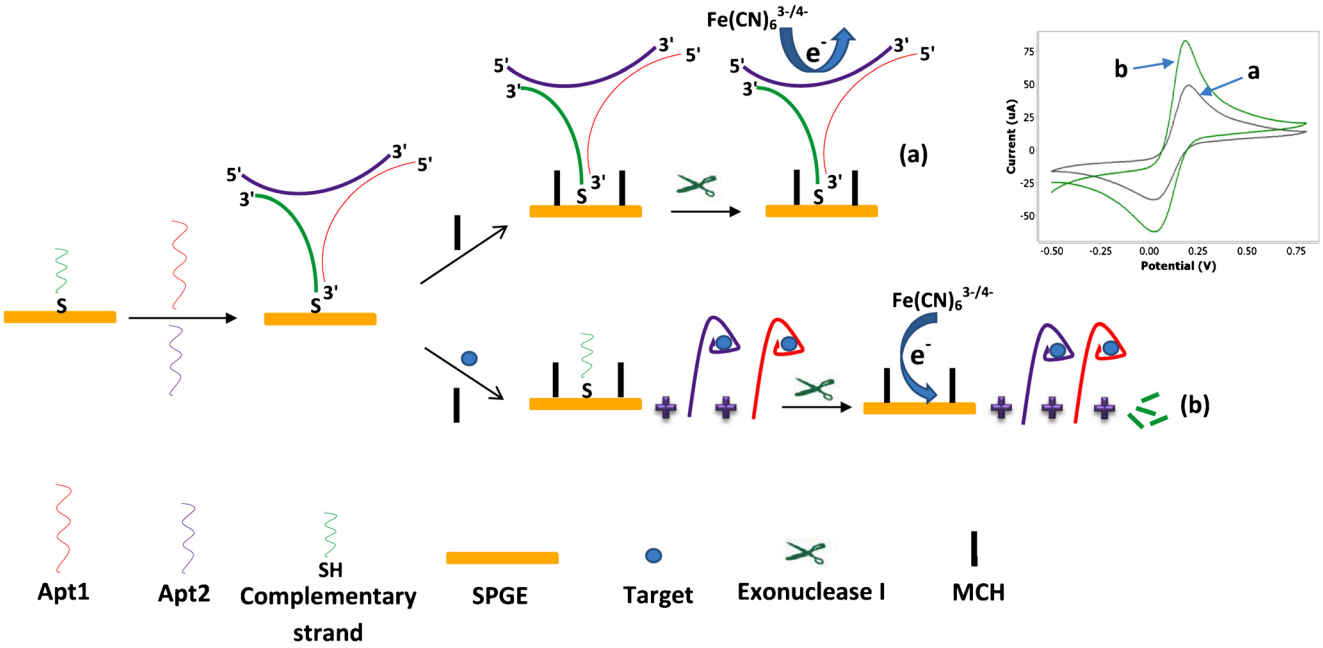

Fig. 17 Myoglobin detection with an electrochemical aptasensor with Y-shaped DNA architecture. Apt aptamer, MCH 6-mercapto-1-hexanol, SPGE screen-printed gold electrode. (Adapted with permission from [81]. Copyright 2016, Elsevier B.V.)

of the strong specific affinity between the aptamer and the target, leading to a reduction of the redox probe electrochemical signals.

A multistep sensing assay for the mycotoxin ochratoxin A was reported by Qing et al. [83] by using an specific aptamer hybridized with a complementary DNA strand previously

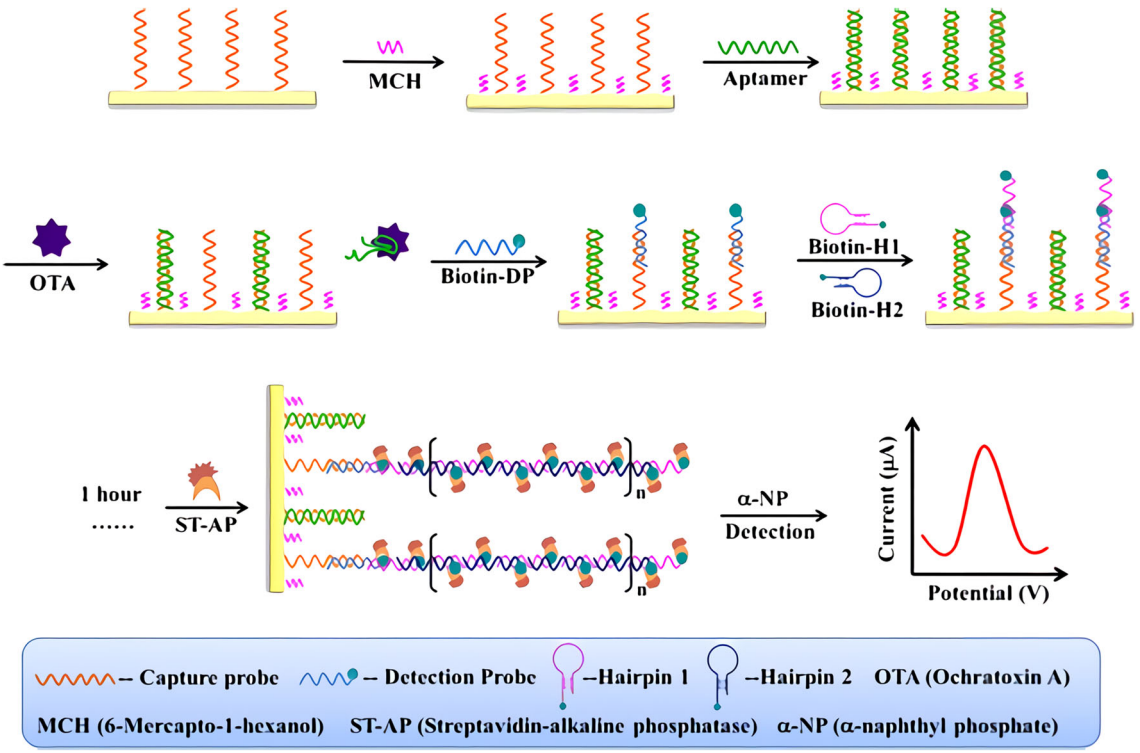

Fig. 18 Sensing strategy for ochratoxin A (OTA) determination using an aptamer-based electrochemical aptasensor with hybridization chain reaction for amplification. DP detection probe, H1 hairpin 1, H2 hairpin 2. (Adapted with permission from [83]. Copyright 2017, Springer-Verlag GmbH, Austria, part of Springer Nature)

immobilized on the surface of a gold electrode (Fig. 18). This assembly was dissociated in the presence of the target, leaving the aptamer–ochratoxin A complex from the electrode. Next, a detection probe and two hairpin-helper DNAs, all end-group functionalized with biotin moieties, were added to promote to the formation of extended double-stranded DNA polymers on the electrode surface through hybridization chain reaction. The electrode was incubated with a streptavidin–alkaline phosphatase conjugate that binds to the remaining biotinylated hairpin DNAs to allow recording of the differential pulse voltammetry signal in the presence of the enzyme substrate. Although this aptasensor showed excellent analytical properties and was validated in cereal samples, the complexity and experimental steps required limit its application for point-of-care determination of the mycotoxin.

Conclusions and remarks

The use of nucleic acid aptamers in electrochemical biosensor technology is a relatively new field in comparison with the use of other affinity and catalytic electroanalytical devices. However, a large number of electrochemical aptasensors have already been developed and successfully used to detect a variety of chemical, biochemical, and biological targets. The growing interest in electrochemical aptasensor technology is based on the structural, functional, and economic advantages of aptamers as affinity biorecognition elements, as well as the possibility to design advanced biosensor architectures and sensing approaches for the fast, simple, sensitive, specific, and in-field detection of low and high molecular weight compounds in real time.

Future advances in this sensing technology should be fueled by progress in SELEX methods that could provide a limitless variety of new bioreceptors with high affinity and selectivity. Biomaterials engineering, microfluidics, nanomaterials chemistry, and nanolithography should also contribute to the tailored design of miniaturized aptasensors with improved functional properties for point-of-care in vitro diagnostics and/or continuous in vivo monitoring of biological biomarkers.

Although electrochemical aptasensors hold much promise as sensing technology, there are several challenges that need to be addressed to produce commercial aptasensor for practical applications. In this sense, further research should be focused on the development of novel strategies aimed at stabilizing the folded aptamer structure in complex media, to increase the sensitivity and reduce the limit of detection without affecting the simplicity of the current sensing approaches, to reduce nonspecific interactions with potential interfering compounds, and to increase resistance of aptamer molecules toward nuclease-catalyzed degradation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

1. Yáñez Sedeño P, Villalonga R, Pingarrón JM. Electroanalytical methods based on hybrid nanomaterials. In: Meyer RA, editor. Encyclopedia of analytical chemistry: applications, theory and instrumentation. Wiley; 2015. p. 1-18.
2. Ronkainen NJ, Halsall HB, Heineman WR. Electrochemical biosensors. Chem. Soc. Rev. 2010;39:1747–63.
3. da Silva ETSG, Souto DEP, Barragan JTC, de F Giarola J, de Moraes ACM, Kubota LT. Electrochemical biosensors in point of care devices: recent advances and future trends. ChemElectroChem. 2017;4:778–94.
4. WiseGuy Reports (2017) Global electrochemical biosensors market trends & forecast 2017 To 2022. Report No. WGR2375992 (https://www.wiseguyreports.com/reports/2375992-global-electrochemical-biosensors-market-trends-forecast-2017-to-2022
5. Kirsch J, Siltanen C, Zhou Q, Revzin A, Simonian A. Biosensor technology: recent advances in threat agent detection and medicine. Chem. Soc. Rev. 2013;42:8733–68.
6. Song Y, Luo Y, Zhu C, Li H, Du D, Lin Y. Recent advances in electrochemical biosensors based on graphene two-dimensional nanomaterials. Biosens. Bioelectron. 2016;76:195–212.
7. Wang L, Xiong Q, Xiao F, Duan H. 2D nanomaterials based electrochemical biosensors for cancer diagnosis. Biosens. Bioelectron. 2017;89:136–51.
8. Wang Z, Dai Z. Carbon nanomaterial-based electrochemical biosensors: an overview. Nanoscale. 2015;7:6420–31.
9. Song S, Wang L, Li J, Fan C, Zhao J. Aptamer-based biosensors. Trends. Anal. Chem. 2008;27:108–17.
10. Plaxco KW, Soh HT. Switch-based biosensors: a new approach towards real-time, in vivo molecular detection. Trends. Biotechnol. 2011;29:1–5.
11. Kaur H, Bruno JG, Kumar A, Sharma TK. Aptamers in the therapeutics and diagnostics pipelines. Theranostics. 2018;8:4016.
12. Razmi N, Baradaran B, Hejazi M, Hasanzadeh M, Mosafer J, Mokhtarzadeh A, et al. Recent advances on aptamer-based biosensors to detection of platelet-derived growth factor. Biosens. Bioelectron. 2018;113:58–71.
13. Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature. 1990;346:818.
14. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990;249:505–10.
15. Colas P, Cohen B, Jessen T, Grishina I, McCoy J, Brent R. Genetic selection of peptide aptamers that recognize and inhibit cyclin-dependent kinase 2. Nature. 1996;380:548.
16. Darmostuk M, Rimpelova S, Gbelcova H, Ruml T. Current approaches in SELEX: An update to aptamer selection technology. Biotechnol. Adv. 2015;33:1141–61.
17. Wu YX, Kwon YJ. Aptamers: The "evolution" of SELEX. Methods. 2016;106:21–8.

18. Bayat P, Nosrati R, Alibolandi M, Rafatpanah H, Abnous K, Khedri M, et al. SELEX methods on the road to protein targeting with nucleic acid aptamers. Biochimie. 2018;154:132–55.
19. Dunn MR, Jimenez RM, Chaput JC. Analysis of aptamer discovery and technology. Nat. Rev. Chem. 2017;1:0076.
20. Cai S, Yan J, Xiong H, Liu Y, Peng D, Liu Z. Investigations on the interface of nucleic acid aptamers and binding targets. Analyst. 2018;143:5317–38.
21. Mayer G. The chemical biology of aptamers. Angew. Chem. 2009;48:2672–89.
22. Iliuk AB, Hu L, Tao WA. Aptamer in bioanalytical applications. Anal. Chem. 2011;83:4440–52.
23. Vermeer AW, Norde W. The thermal stability of immunoglobulin: unfolding and aggregation of a multi-domain protein. Biophys. J. 2000;78:394–404.
24. Nutiu R, Li Y. Structure-switching signaling aptamers. J. Am. Chem. Soc. 2003;125:4771–8.
25. Xiao Y, Lai RY, Plaxco KW. Preparation of electrode-immobilized, redox-modified oligonucleotides for electrochemical DNA and aptamer-based sensing. Nat. Protoc. 2007;2:2875–80.
26. Cheng AK, Sen D, Yu HZ. Design and testing of aptamer-based electrochemical biosensors for proteins and small molecules. Bioelectrochemistry. 2009;77:1–12.
27. Schoukroun-Barnes LR, Macazo FC, Gutierrez B, Lottermoser J, Liu J, White RJ. Reagentless, structure-switching, electrochemical aptamer-based sensors. Annu. Rev. Anal. Chem. 2016;9:163–81.
28. Vallée-Bélisle A, Plaxco KW. Structure-switching biosensors: inspired by nature. Curr. Opin. Struct. Biol. 2010;20:518–26.
29. Mao Y, Luo C, Ouyang Q. Studies of temperature-dependent electronic transduction on DNA hairpin loop sensor. Nucleic Acids Res. 2003;31:108.
30. Hianik T, Ostatná V, Sonlajtnerova M, Grman I. Influence of ionic strength, pH and aptamer configuration for binding affinity to thrombin. Bioelectrochemistry. 2007;70:127–33.
31. Pilehvar S, Jambrec D, Gebala M, Schuhmann W, De Wael K. Intercalation of proflavine in ssDNA aptamers: effect on binding of the specific target chloramphenicol. Electroanalysis. 2015;27:1836–41.
32. Kékedy-Nagy L, Shipovskov S, Ferapontova EE. Effect of a dual charge on the DNA-conjugated redox probe on DNA sensing by short hairpin beacons tethered to gold electrodes. Anal. Chem. 2016;88:7984–90.
33. Sachan A, Ilgu M, Kempema A, Kraus GA, Nilsen-Hamilton M. Specificity and ligand affinities of the cocaine aptamer: impact of structural features and physiological NaCl. Anal. Chem. 2016;88:7715–23.
34. Chen Y, Pui TS, Kongsuphol P, Tang KC, Arya SK. Aptamer-based array electrodes for quantitative interferon-γ detection. Biosen. Bioelectron. 2014;53:257–62.
35. Yu ZG, Lai RY. A reagentless and reusable electrochemical aptamer-based sensor for rapid detection of ampicillin in complex samples. Talanta. 2018;176:619–24.
36. Das R, Dhiman A, Mishra SK, Haldar S, Sharma N, Bansal A, et al. Structural switching electrochemical DNA aptasensor for the rapid diagnosis of tuberculous meningitis. Int. J. Nanomed. 2019;14:2103.
37. Liu Y, Matharu Z, Rahimian A, Revzin A. Detecting multiple cell-secreted cytokines from the same aptamer-functionalized electrode. Biosens. Bioelectron. 2015;64:43–50.
38. Jia J, Feng J, Chen HG, Luo HQ, Li NB. A simple electrochemical method for the detection of ATP using target-induced conformational change of dual-hairpin DNA structure. Sens. Actuators B. 2016;222:1090–5.
39. Pividori MI, Merkoci A, Alegret S. Electrochemical genosensor design: immobilisation of oligonucleotides onto transducer surfaces and detection methods. Biosens. Bioelectron. 2000;15:291–303.
40. Tabrizi MA, Shamsipur M, Saber R, Sarkar S, Besharati M. An electrochemical aptamer-based assay for femtomolar determination of insulin using a screen printed electrode modified with mesoporous carbon and 1,3,6,8-pyrenetetrasulfonate. Microchim. Acta. 2018;85:59.
41. Yu Z, Luan Y, Li H, Wang W, Wang X, Zhang Q. A disposable electrochemical aptasensor using single-stranded DNA-methylene blue complex as signal-amplification platform for sensitive sensing of bisphenol A. Sens. Actuators B. 2019;284:73–80.
42. Roushani M, Shahdost-fard F. Covalent attachment of aptamer onto nanocomposite as a high performance electrochemical sensing platform: fabrication of an ultra-sensitive ibuprofen electrochemical aptasensor. Mater. Sci. Eng. 2016;68:128–35.
43. Wei M, Zhang W. The determination of ochratoxin A based on the electrochemical aptasensor by carbon aerogels and methylene blue assisted signal amplification. Chem. Cent. J. 2018;12:45.
44. Raouafi A, Sánchez A, Raouafi N, Villalonga R. Electrochemical aptamer-based bioplatform for ultrasensitive detection of prostate specific antigen. Sens. Actuators B. 2019;297:126762.
45. Karimipour M, Heydari-Bafrooei E, Sanjari M, Johansson MB, Molaei M. A glassy carbon electrode modified with TiO_2(200)-rGO hybrid nanosheets for aptamer based impedimetric determination of the prostate specific antigen. Microchim. Acta. 2019;186:33.
46. Arya SK, Zhurauski P, Jolly P, Batistuti MR, Mulato M, Estrela P. Capacitive aptasensor based on interdigitated electrode for breast cancer detection in undiluted human serum. Biosens. Bioelectron. 2018;102:106–12.
47. Grabowska I, Sharma N, Vasilescu A, Iancu M, Badea G, Boukherroub R, et al. Electrochemical aptamer-based biosensors for the detection of cardiac biomarkers. ACS Omega. 2018;3:12010–8.
48. Fenzl C, Nayak P, Hirsch T, Wolfbeis OS, Alshareef HN, Baeumner AJ. Laser-scribed graphene electrodes for aptamer-based biosensing. ACS Sens. 2017;2:616–20.
49. Singh NK, Thungon PD, Estrela P, Goswami P. Development of an aptamer-based field effect transistor biosensor for quantitative detection of Plasmodium falciparum glutamate dehydrogenase in serum samples. Biosens. Bioelectron. 2019;123:30–5.
50. Argoubi W, Sánchez A, Parrado C, Raouafi N, Villalonga R. Label-free electrochemical aptasensing platform based on mesoporous silica thin film for the detection of prostate specific antigen. Sens. Actuators B. 2018;255:309–15.
51. Jolly P, Tamboli V, Harniman RL, Estrela P, Allender CJ, Bowen JL. Aptamer-MIP hybrid receptor for highly sensitive electrochemical detection of prostate specific antigen. Biosens. Bioelectron. 2016;75:188–95.
52. Wang YH, Xia H, Huang KJ, Wu X, Ma YY, Deng R, et al. Ultrasensitive determination of thrombin by using an electrode modified with WSe_2 and gold nanoparticles, aptamer-thrombin-aptamer sandwiching, redox cycling, and signal enhancement by alkaline phosphatase. Microchim. Acta. 2018;185:502.
53. Kim SH, Nam O, Jin E, Gu MB. A new coccolith modified electrode-based biosensor using a cognate pair of aptamers with sandwich-type binding. Biosens. Bioelectron. 2019;123:160–6.
54. Seo HB, Gu MB. Aptamer-based sandwich-type biosensors. J. Biol. Eng. 2017;11:11.
55. Shui B, Tao D, Cheng J, Mei Y, Jaffrezic-Renault N, Guo Z. A novel electrochemical aptamer-antibody sandwich assay for the detection of tau-381 in human serum. Analyst. 2018;143:3549–54.
56. Lee S, Hayati S, Kim S, Lee HJ. Determination of protein tyrosine kinase-7 concentration using electrocatalytic reaction and an aptamer-antibody sandwich assay platform. Catal. Today. 2019. https://doi.org/10.1016/j.cattod.2019.05.029.
57. Chung S, Moon JM, Choi J, Hwang H, Shim YB. Magnetic force assisted electrochemical sensor for the detection of thrombin with aptamer-antibody sandwich formation. Biosens. Bioelectron. 2018;117:480–6.

58. Wang QL, Cui HF, Song X, Fan SF, Chen LL, Li MM, et al. A label-free and lectin-based sandwich aptasensor for detection of carcinoembryonic antigen. Sens. Actuators B. 2018;260:48–54.
59. Paniagua G, Villalonga A, Eguílaz M, Vegas B, Parrado C, Rivas G, et al. Amperometric aptasensor for carcinoembryonic antigen based on the use of bifunctionalized Janus nanoparticles as biorecognition-signaling element. Anal. Chim. Acta. 2019;1061: 84–91.
60. Jiang B, Li F, Yang C, Xie J, Xiang Y, Yuan R. Target-induced catalytic hairpin assembly formation of functional Y-junction DNA structures for label-free and sensitive electrochemical detection of human serum proteins. Sens. Actuators B. 2017;244:61–6.
61. Shi K, Dou B, Yang J, Yuan R, Xiang Y. Target-triggered catalytic hairpin assembly and TdT-catalyzed DNA polymerization for amplified electronic detection of thrombin in human serums. Biosens. Bioelectron. 2017;87:495–500.
62. Zhang R, Gu Y, Wang Z, Li Y, Fan Q, Jia Y. Aptamer cell sensor based on porous graphene oxide decorated ion-selective-electrode: double sensing platform for cell and ion. Biosens. Bioelectron. 2018;117:303–11.
63. Aktas GB, Skouridou V, Masip L. Sandwich-type aptasensor employing modified aptamers and enzyme-DNA binding protein conjugates. Anal.Bioanal. Chem. 2019; 411:3581.
64. Chen X, Wang Y, Zhang Y, Chen Z, Liu Y, Li Z, et al. Sensitive electrochemical aptamer biosensor for dynamic cell surface N-glycan evaluation featuring multivalent recognition and signal amplification on a dendrimer–graphene electrode interface. Anal. Chem. 2014;86:4278–86.
65. Shangguan D, Li Y, Tang Z, Cao ZC, Chen HW, Mallikaratchy P, et al. Aptamers evolved from live cells as effective molecular probes for cancer study. Proc. Natl. Acad. Sci. U. S. A. 2006;103: 11838–43.
66. Kiernan JA. Localization of α-D-glucosyl and α-D-mannosyl groups of mucosubstances with concanavalin A and horseradish peroxidase. Histochemistry. 1975;44:39–45.
67. Abbaspour A, Norouz-Sarvestani F, Noori A, Soltani N. Aptamer-conjugated silver nanoparticles for electrochemical dual-aptamer-based sandwich detection of Staphylococcus aureus. Biosens. Bioelectron. 2015;68:149–55.
68. Brosel-Oliu S, Ferreira R, Uria N, Abramova N, Gargallo R, Muñoz-Pascual FX, et al. Novel impedimetric aptasensor for label-free detection of Escherichia coli O157: H7. Sens. Actuators B. 2018;255:2988–95.
69. Li Z, Yin J, Gao C, Sheng L, Meng A. A glassy carbon electrode modified with graphene oxide, poly (3, 4-ethylenedioxythiophene), an antifouling peptide and an aptamer for ultrasensitive detection of adenosine triphosphate. Microchim. Acta. 2019;186:90.
70. Eissa S, Siaj M, Zourob M. Aptamer-based competitive electrochemical biosensor for brevetoxin-2. Biosens. Bioelectron. 2015;69:148–54.
71. Kazane I, Gorgy K, Gondran C, Spinelli N, Zazoua A, Defrancq E, et al. Highly sensitive bisphenol-A electrochemical aptasensor based on poly(pyrrole-nitrilotriacetic acid)-aptamer film. Anal. Chem. 2016;88:7268–73.
72. Wu M, Kempaiah R, Huang PJJ, Maheshwari V, Liu J. Adsorption and desorption of DNA on graphene oxide studied by fluorescently labeled oligonucleotides. Langmuir. 2011;27:2731–8.
73. Liu B, Sun Z, Zhang X, Liu J. Mechanisms of DNA sensing on graphene oxide. Anal. Chem. 2013;85:7987–93.
74. Park JS, Goo NI, Kim DE. Mechanism of DNA adsorption and desorption on graphene oxide. Langmuir. 2014;30:12587–95.
75. Ahour F, Ahsani MK. An electrochemical label-free and sensitive thrombin aptasensor based on graphene oxide modified pencil graphite electrode. Biosens. Bioelectron. 2016;86:764–9.
76. Eissa S, Zourob M. In vitro selection of DNA aptamers targeting β-lactoglobulin and their integration in graphene-based biosensor for the detection of milk allergen. Biosens. Bioelectron. 2017;91:169–74.
77. Yu SH, Lee CS, Kim TH. Electrochemical detection of ultratrace lead ion through attaching and detaching DNA aptamer from electrochemically reduced graphene oxide electrode. Nanomaterials. 2019;9:817.
78. Aceta Y, Del Valle M. Graphene electrode platform for impedimetric aptasensing. Electrochim. Acta. 2017;229:458–66.
79. Jain P, Das S, Chakma B, Goswami P. Aptamer-graphene oxide for highly sensitive dual electrochemical detection of Plasmodium lactate dehydrogenase. Anal. Biochem. 2016;514:32–7.
80. Stephanopoulos N, Freeman R. DNA-based materials as self-assembling scaffolds for interfacing with cells. In: Azevedo HS, da Silva RMP, editors. Self-assembling biomaterials. Elsevier; 2018, p. 157-175.
81. Taghdisi SM, Danesh NM, Ramezani M, Emrani AS, Abnous K. A novel electrochemical aptasensor based on Y-shape structure of dual-aptamer-complementary strand conjugate for ultrasensitive detection of myoglobin. Biosens. Bioelectron. 2016;80:532–7.
82. Wang D, Xiao X, Xu S, Liu Y, Li Y. Electrochemical aptamer-based nanosensor fabricated on single Au nanowire electrodes for adenosine triphosphate assay. Biosens. Bioelectron. 2018;99:431–7.
83. Qing Y, Li X, Chen S, Zhou X, Luo M, Xu X, et al. Differential pulse voltammetric ochratoxin A assay based on the use of an aptamer and hybridization chain reaction. Microchim. Acta. 2017;184:863–70.