Aptamer-based biosensors

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Nucleic-acid aptamers have attracted intense interest and found wide applications in a range of areas. In this review, we summarize recent advances in the development of aptamer-based biosensors and bioassay methods, most of which have employed electrochemical, optical and mass-sensitive analytical techniques. Aptamers exhibit many advantages as recognition elements in biosensing when compared to traditional antibodies. They are small in size, chemically stable and cost effective. More importantly, aptamers offer remarkable flexibility and convenience in the design of their structures, which has led to novel biosensors that have exhibited high sensitivity and selectivity. Recently, the combination of aptamers with novel nanomaterials has significantly improved the performance of aptamer-based sensors, which we also review in this article. In view of the unprecedented advantages brought by aptamers, we expect aptamer-based biosensors to find broad applications in biomedical diagnostics, environmental monitoring and homeland security.

Keywords: Aptamer; Biosensor; Nanomaterial; Nanotechnology

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1. Introduction

Natural selection is one of the basic mechanisms of evolution, which was discovered by Darwin over 150 years ago. However, the importance of artificial, in-vitro selection was recognized much later.

Aptamers are an excellent example of functional molecules selected in vitro. In 1990, two groups independently developed in-vitro selection and amplification for the isolation of RNA sequences that could specifically bind to target molecules [1,2]. These functional RNA oligonucleotides were then termed *aptamers*, derived from the Latin *aptus*, meaning ''to fit'' [3]. Later, DNA-based aptamers were also found [4].

Since its discovery, aptamer technology has received tremendous attention in scientific and industrial communities. After nearly 20 years' endeavor, DNA and RNA aptamers have been identified as binding tightly to a broad range of targets (e.g., proteins, peptides, amino acids, drugs, metal ions and even whole cells), especially with the development of rapid, automated, selection technologies [5]. Aptamers often possess high affinity for their targets, which is derived from their capability of folding upon binding with their target molecule (i.e. they can either incorporate small molecules into their nucleic acid structure or be integrated into the structure of macromolecules (e.g., proteins [6])).

Aptamers have become increasingly important molecular tools for diagnostics and therapeutics. In particular, aptamer-based biosensors possess unprecedented advantages compared to biosensors using natural receptors such as antibodies and enzymes:

- First, aptamers with high specificity and affinity can in principle be selected in vitro for any given target, ranging from small molecules to large proteins and even cells, thus making it possible to develop a wide range of aptamer-based biosensors.
- Second, aptamers, once selected, can be synthesized with high reproducibility and purity from commercial sources. Also, in contrast to protein-based antibodies or enzymes, DNA aptamers are usually highly chemically stable.
- Third, aptamers often undergo significant conformational changes upon target binding. This offers great flexibility in design of novel biosensors with high detection sensitivity and selectivity.

In recent years, in-depth understanding of nucleic-acid aptamers in terms of their conformational and ligand-binding properties has produced intense interest, and led to a range of bioassay methods that rely on aptamer receptors [7–10]. In line with this trend, below we review recent research advances of aptamer-based sensors employing electrochemical, optical and mass-sensitive transducers.

2. Targets

Aptamers are also termed ''chemical antibodies'' because of their artificial process

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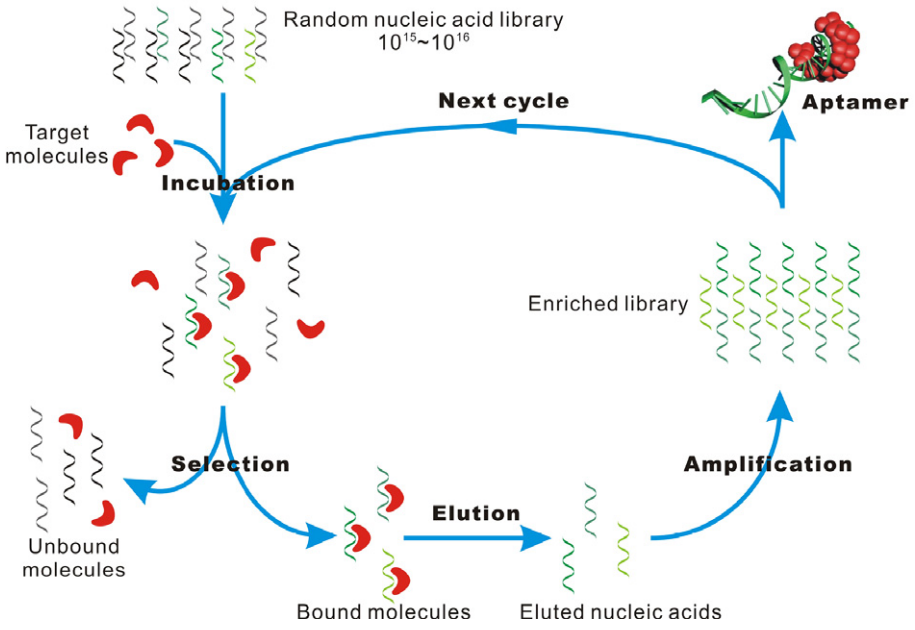

Figure 1. Scheme for the Systematic Evolution of Ligands by EXponential (SELEX) enrichment process. A random nucleic acid library is incubated with a target molecule, and unbound molecules are separated from bound molecules. Bound nucleic acids are eluted, amplified by PCR (polymerase chain reaction) and serve as an enriched library for the next cycle. For every target, 6–12 consecutive cycles are performed and the final enriched library is cloned and sequenced.

in vitro based on Systematic Evolution of Ligands by EXponential enrichment (SELEX) (Fig. 1). Unlike the preparation of antibodies, which relies on induction of an animal immune system, the SELEX process enables the fabrication of aptamers for non-immunogenic and toxic targets that it is otherwise impossible to obtain by the immune system [3]. Moreover, it is also possible to produce aptamers to specific regions of targets, which is sometimes difficult for antibodies, since the animal-immune system inherently contains epitopes on target molecules. Until now, aptamers have been selected toward a broad range of targets, including metal ions (e.g., K^+, Hg^{2+} and Pb^{2+}), small organic molecules (e.g., amino acids, ATP, antibiotics, vitamins and cocaine) organic dyes, peptides and proteins (e.g., thrombin, growth factors and HIV-associated peptides) and even whole cells or microorganisms (e.g., bacteria) [11–22]. Importantly, the availability of such a large pool of aptamers makes it possible to develop novel bioassay tools covering areas that include diagnostics, anti-bioterrorism, and environmental and food analysis [5].

Aptamers often possess high selectivity and affinity toward their targets. Analogous to that of antigens/antibodies, interactions between aptamers and their molecular targets are usually so specific that even small variations in the target molecule may disrupt aptamer binding (e.g., the aptamers for theophylline and L-arginine can discriminate closely related chemical structures by factors as high as 4 orders of magnitude [23,24]). In addition to this high selectivity, aptamers bind to their targets with high affinity, particularly with macromolecules (e.g., proteins), which often possess remarkable dissociation constants (K_d) ranging from picomolar to nanomolar [23].

3. Assay configuration

Analogous to immunoassays based on the antigen-antibody interaction, aptamer-based bioassays can adopt different assay configurations to transduce bio-recognition events. Since aptamers have been selected to bind very different targets, ranging from small molecules to macromolecules, such as proteins, various assay configurations have been designed and reported. Nevertheless, the majority of these designs fall into two categories of configuration (Fig. 2):

- single-site binding; and,
- dual-site binding.

The design for sensors largely relies on the inherently different recognition modes of each aptamer-target pair [6]. For small molecular targets, nuclear magnetic resonance (NMR) studies have indicated that they are often buried within the binding pockets of aptamer structures (Fig. 2A), leaving little room for the interaction with a second molecule. Because of this limitation, small-molecule targets are often assayed using the single-site binding configuration. By contrast, protein targets are structurally complicated, allowing the interplay of various discriminatory contacts (e.g., stacking, shape

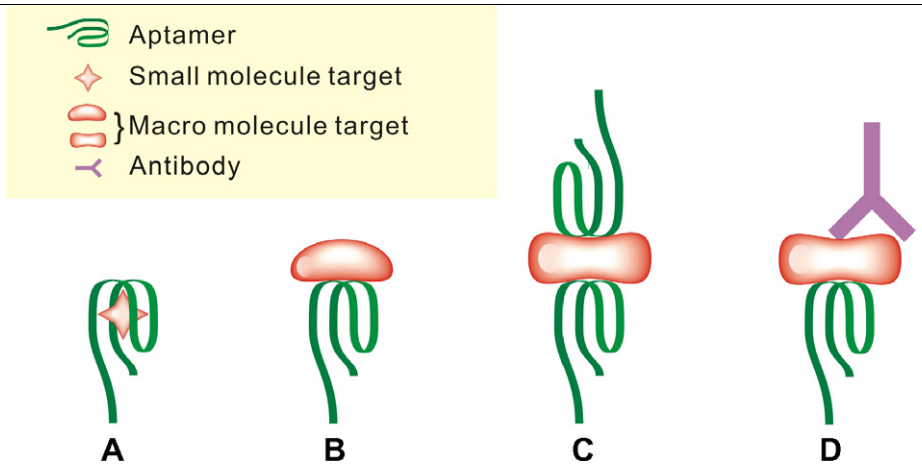

Figure 2. Aptamer-based assay formats. (A) Small-molecule target buried within the binding pockets of aptamer structures; (B) single-site format; (C) dual-site (sandwich) binding format with two aptamers; and, (D) ''sandwich'' binding format with an aptamer and an antibody.

complementarity, electrostatic interactions, and hydrogen bonding). As a result, protein targets can be assayed via both single-site binding (Fig. 2B) and dual-site binding (Fig. 2C). Of note, dual-site binding also relies on the availability of a pair of aptamers that bind to different regions of the protein.

The dual-site binding assay, also known as the ''sandwich'' assay, is one of the most used assay formats. In this approach, the analyte is sandwiched by a pair of aptamers (Fig. 2C), one capture probe and the other reporter probe. Capture probes are often immobilized on the surface of solid supports (e.g., electrodes, glass chips, nanoparticles or micro-particles), while reporter probes are often conjugated with signaling moieties (e.g., fluorophores, enzymes or nanoparticles (NPs)). Generally speaking, capture and reporter probes have different nucleic acid sequences; however, in limited cases, some proteins (e.g., dimeric) contain two identical binding sites, thus allowing the use of a single aptamer for the sandwich assay. Also of note, in cases when there are no two aptamers sharing identical or overlapping binding sites on the target of interest, it is possible to use an antibody as the second ''aptamer'' (Fig. 2D). Apparently, this also highlights the importance of identifying multiple aptamers for a single molecular target.

4. Assay formats

Along with the rapid progress of modern analytical technologies and the application of novel analytical reagents (e.g., nanomaterial-based probes), more and more aptamer-based bioassay formats have been developed in recent years. Aptamer-based sensors (aptasensors) have attracted particular attention.

Apart from the inherent advantages of biosensors (e.g., no need for additional processing steps), aptamer-based biosensors offer the advantage of reusability over antibodies. Furthermore, their small size and versatility allow efficient immobilization at high density, which is of vital importance in multiplexing miniaturized systems (e.g., bioarrays or biochips). In this review, aptamer-based bioassays are classified as electrochemical, optical and mass-sensitive, according to their signal-harvesting method.

4.1. Electrochemical

Nucleic acids aptamers fold their flexible, single-stranded chains into well-defined three-dimensional (3D) structures upon binding to their target molecules, so, provided aptamers are immobilized on a conducting support, this behavior enabled redox-active moieties to be tethered to them and identification of the formation of the aptamer–target complexes by probing the electron-transfer features of the redox moieties of the rigidified 3D complexes. Several electrochemical aptasensors have been developed based on this strategy.

In line with the conformational transition of the anti-thrombin aptamer upon binding to thrombin, an electrochemical thrombin aptasensor was constructed by immobilizing an aptamer labeled with a redox-active methylene blue (MB) on an electrode [25]. The flexible conformation of the aptamer enabled the electrical communication of MB with the electrode. After binding with thrombin, the aptamer self-assembled into a G-quadruplex structure and shielded MB from electron-transfer communication with the electrode (Fig. 3A). This sensing format has a disadvantage because of a negative signal (i.e. the amperometric response decreases as a result of the association of target thrombin with the aptamer).

To circumvent this problem, several signal-on aptosensors were later developed. One approach employed a bifunctionalized anti-thrombin aptamer tagged with a

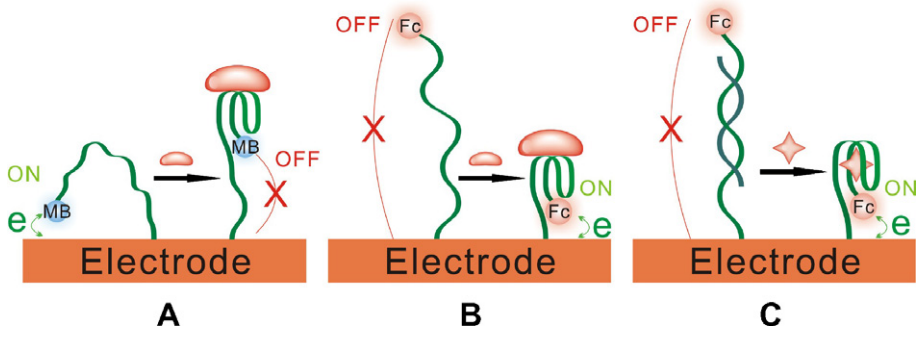

Figure 3. Schemes for ''signal-off'' and ''signal-on'' electrochemical sensors. (A) After binding to thrombin, the aptamer probe self-assembles into a G-quadruplex structure and shields MB from electron-transfer communication with the electrode, leading to a negative signal [25]; (B) formation of a complex of thrombin and the aptamers makes the G-quadruplex configuration rigid and results in the orientation of the ferrocene units in the proximity of the electrode, leading to easy electron transfer between the electro-active ferrocene units and the electrode and producing a positive signal [26–28]; and, (C) the presence of ATP unties the rigid DNA duplex and liberates the complementary sequence, while making the aptamer sequence form a rigid 3D structure. This brings the ferrocene tag to the proximity of the electrode surface and turns on electron transfer, producing positive signals [32].

terminal electroactive ferrocene group as the redox label and a thiol group at the other terminus [26–28]. The long, flexible, modified aptamer chain prevented electrical contact of the ferrocene label with the electrode. The formation of a complex of thrombin and the aptamers made the G-quadruplex aptamer configuration rigid and resulted in the orientation of the ferrocene units towards the electrode (Fig. 3B). This led to electron-transfer between the electro-active ferrocene units and the electrode, and produced a positive signal in the presence of thrombin. In a related approach, a distinct fold change of the modified aptamer was employed [29,30]. In the absence of targets (either cocaine or platelet-derived growth factor, PDGF), the immobilized aptamer chain remained partially unfolded, with only one of its three double-stranded stems intact. In the presence of targets, the aptamer folded into the target-specific three-way junction, which shortened the electron-transfer distance and increased the signal.

In the two electrochemical sensors mentioned above, the aptamers stayed in a relatively soft conformation, which led to high background and hence low signal-to-noise ratio. An improved approach relied on the use of DNA-duplex probes assembled on gold (Au) electrodes [31,32].

In one of these formats, the DNA structure comprised two double-stranded domains separated by a non-complementary nucleic-acid bridge. The upper domain was formed by an anti-thrombin aptamer sequence with the MB-tagged complementary sequence. In the presence of thrombin, it induced the formation of the G-quadruplex structure, liberating the MB-tagged chain that could freely contact and communicate electrons with the electrode [31].

The other format employed a DNA-duplex probe comprising an anti-ATP aptamer sequence labeled with ferrocene and its complementary sequence. In the presence of ATP, the complementary sequence was liberated while the aptamer sequence formed a rigid 3D structure with the aid of ATP. This transition brought the ferrocene tag to the proximity of the electrode surface, which turned on the electron transfer and produced electrochemical signals [32] (Fig. 3C). This sensor could detect ATP at a wide concentration range (10 nM–1 mM) with high sensitivity.

Other assay formats employed redox-active reporting units that were not covalently tethered to aptamers. These formats provided a ''label-free'' method to electrochemically monitor the aptamer–target interaction. A related approach used intercalative MB to detect thrombin. MB was intercalated into the double-stranded DNA domain of a hairpin configuration that contained the anti-thrombin aptamer [33](Fig. 4A). The binding of thrombin with the aptamer unit opened the hairpin structure, thus releasing the intercalated MB. As a result, the amperometric response decreased in line with the addition of thrombin.

Other related approaches employed either cationic redox-active reporting units (ferrocene-functionalized polyelectrolyte poly(3-alkoxy-4-methylthiophene) [34] or $[Ru(NH_3)_6]^{3+}$[35]) that were bound to the electrode via electrostatic interaction with the DNA aptamer phosphate backbone (Fig. 4B). These interactions between redox reporters and immobilized aptamers yielded prominent voltammetric responses. The binding of either thrombin or lysozyme with their respective aptamers blocked the binding of the cationic reporting units and depleted their electrochemical response. Apparently, the disadvantage of the format is a negative detection signal for analyzing targets and the unsatisfactory limit of detection (LOD) (only 10^{-6} μM for thrombin or 0.5 μg/mL for lysozyme). An improved assay format used the

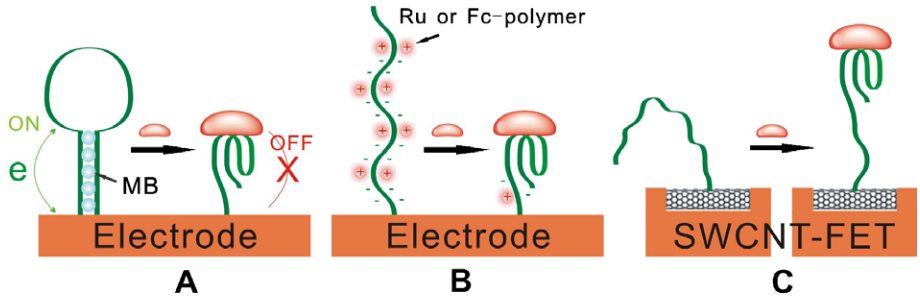

Figure 4. Label-free electrochemical aptasensors. (A) Binding the target with the aptamer opens the hairpin structure, thus releasing the intercalated MB and decreasing electronic signals [33]; (B) binding targets with their aptamers blocked the binding of the cationic reporting units and depleted their electrochemical response [34,35]; and, (C) in the SWCNT-FET sensor, binding target with the aptamer altered the conductance through the device, thus enabling the detection of targets [36,37].

same cationic redox-active polymer (ferrocene-functionalized polyelectrolyte poly(3-alkoxy- 4-methylthiophene) and a peptide nucleic acid (PNA) linked to an Au electrode. After interaction of anti-thrombin aptamer and thrombin in solution, the resulting mixture was treated with an S1 nuclease to digest all free nucleic acid. Then, with thermal treatment, the intact aptamer protected by the thrombin was dissociated and hybridized with the PNA. This resulted in the negatively-charged duplex structure that binds the cationic redox-active polymer, which enhanced the voltammetric responses of the electrode in line with increasing thrombin concentration. The assay format enabled the analysis of thrombin with a low LOD (10 nM), which nevertheless required many assay steps.

The application of nanomaterials provides a novel approach to develop really label-free, high-sensitivity sensors. For example, a single-walled carbon nanotube field-effect transistor (SWCNT-FET) device was fabricated to monitor aptamer–protein affinity-binding processes [36]. The merit of using aptamers in FET-type sensing-assay formats lies in their small size. It is difficult to monitor protein–protein binding processes in immunological FETs (ImmunoFETs), since recognition might occur outside the electrical double-layer associated with the gate, resulting in small potential changes on the gate. This is because the size of antibodies (≈10 nm) is much larger than the electrical double layer so that most of the protein charges will be at a distance greater than the Debye length (≈3 nm in 10 mM ionic concentration). However, the dimensions of aptamers (1–2 nm) enable the perturbation of the gate potential by proteins that link to the aptamers, as the recognition binding events occur within the Debye length of the double layer. Accordingly, SWCNTs were assembled between source and drain electrodes, and the aptamer against thrombin was assembled on the CNTs. The binding of thrombin to the aptamer altered conductance through the device, thus enabling detection of the protein (Fig. 4C). Conductance through the device was specific for thrombin, while non-cognate protein (e.g., elastase) had little effect on conductance.

In another similar assay format [37], an aptamer-modified CNT-FET was compared to an antibody-modified one. The comparison showed that the performance of the aptamer-modified CNT-FET provided better results than those obtained from the antibody-modified one under identical conditions. Importantly, the aptamer-modified CNT-FET could detect immunoglobulin E (IgE) at an LOD as low as 250 pM.

In order to obtain high specificity and introduce amplification factors to improve sensitivity, sandwich configurations are often adopted in aptamer-based electrochemical bioassays. Usually, the biocatalytic properties of enzymes are used to detect and to amplify the analysis of targets with their aptamers. For example, glucose dehydrogenase (GDH) was employed as a biocatalytic label for the amplified amperometric detection of thrombin [38]. Thrombin was linked to a 15-mer thiolated aptamer linked to an Au electrode, and the GDH–avidin conjugate was linked to the surface by its association to the biotinylated 29-mer aptamer bound to the thrombin. The bioelectrocatalyzed oxidation of glucose in diffusional mediator enabled the amperometric detection of the thrombin.

In an improved approach [39], aptamer-functionalized Pt NPs (PtNPs) were employed as catalytic labels instead of enzymes (Fig. 5A). The PtNPs catalyzed the electrochemical reduction of H_2O_2 and amplified detection of thrombin with an LOD of nM. This assay format is very similar to the biocatalytic sandwich-type analysis using GDH but improved the LOD by a factor of 80-fold.

Besides acting as catalysts, NPs (e.g., AuNPs) have also been employed as carriers for ultrasensitive electrochemical detection of proteins [40]. In this format, AuNPs were functionalized with anti-thrombin aptamers containing poly-adenine (poly-A) sequence. They bound to the thrombin captured by immobilized anti-thrombin antibody (Fig. 5B). The adenine nucleobases were released by acid or nuclease degradation and were directly

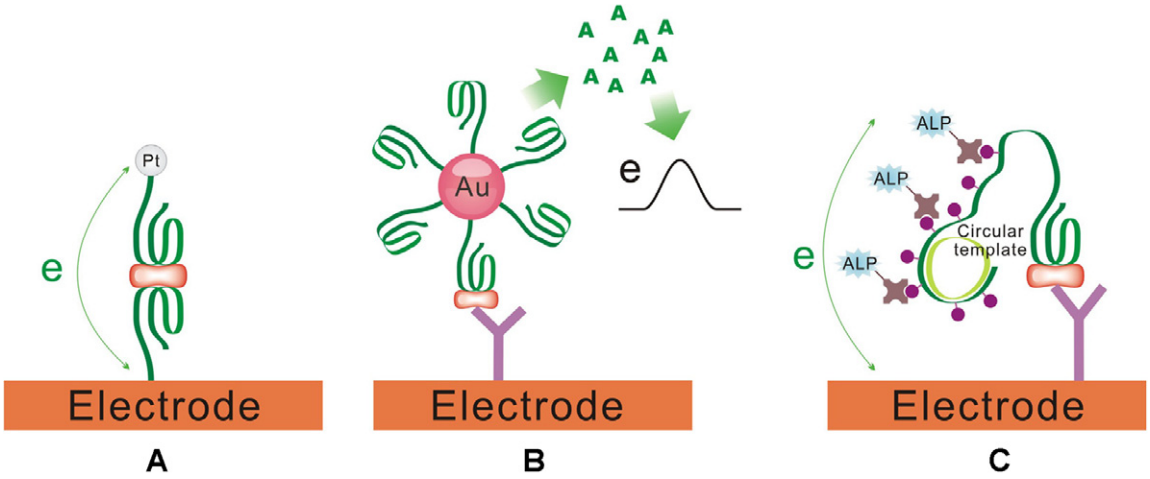

Figure 5. ''Sandwich''-type aptamer-based electrochemical sensors with signal amplification. (A) Aptamer-functionalized PtNPs were employed as catalytic labels to catalyze the electrochemical reduction of H_2O_2 and enabled the amplified detection of targets [39]; (B) AuNPs functionalized with aptamers containing poly-A were used as reporting probes. The adenine nucleobases released from them were directly detected to produce amplified signals [40]; and, (C) acting as the reporter, the aptamer-primer sequence mediated an in-situ RCA reaction, leading to significant enhancement in detection sensitivity [41].

detected using a pyrolytic graphite electrode. Because one NP carried a large number of aptamers, the thrombin-binding process was substantially amplified, which led to a low LOD of 0.1 ng/mL.

In another sandwich-type electrochemical bioassay, instead of using carriers, the aptamers were designed to allow rolling circle amplification (RCA) [41]. As a result, the aptamer-primer sequence mediated an in-situ RCA reaction that generated hundreds of copies of the circular DNA template, leading to significant enhancement in the detection sensitivity (Fig. 5C). This assay strategy produced a wide detection range of four orders of magnitude and an LOD as low as 10 fM. Moreover, the novel aptamer-primer design circumvented time-consuming preparation of the antibody-DNA conjugate in conventional immuno-RCA assays.

4.2. Optical

Aptamers have also been widely used as bio-recognition elements in optical bioassays. Of these assay formats, fluorescence and colorimetry are the two most popular techniques.

Fluorescent detection is widely employed due to the ease of labeling aptamers with fluorescent dyes, the availability of many different fluorophores and quenchers, and the inherent capability for real-time detection. Several main strategies have been developed for converting aptamers into fluorescent signaling probes that are often called signaling aptamers (Fig. 6). A frequently adopted format is an aptamer-based molecular beacon (aptabeacon), which was a modified version of traditional molecular beacons [42]. It places an aptamer sequence in a molecular beacon-like, hairpin structure, end-labeled with a fluorophore and a quencher. The binding of the target disrupts the stem, separating the fluorophore from the quencher and leading to fluorescence signals (Fig. 6A). Another frequently adopted format places an fluorophore-labeled aptamer in a duplex structure with a complementary DNA sequence labeled with a quencher; the presence of targets forces the departure of the complementary strand from the aptamer, accompanied by an increase in fluorescence [43,44] (Fig. 6B). To date, the above fluorescence-quenching-based assay formats have allowed only qualitative or semi-quantitative bioassays.

Recently, instead of using organic fluorescent dyes, quantum dots (QDs) have been employed to improve their assay performance [45,46] and to detect drug delivery in cells [47]. An alternative format is the use of fluorescence resonance transfer (FRET), which relies on the energy transfer between two fluorescent molecules – donor and acceptor. However, despite the great efforts currently being made to optimize dual-labeled oligonucleotides, little progress has been made on a quantification probe using the FRET technique. This is probably because the improvement in FRET depends on many factors (e.g., nature of the dyes, spacer length or dye–dye interactions). More importantly, fluorescence-quenching-based or FRET-based probes are difficult to apply directly to analyzing targets in their native environments because of the interference of background signal.

In a new development, a wavelength-shifting aptamer was designed for rapid, sensitive detection of PDGF [48]. Labeled with one pyrene at each end, the aptamer switches its fluorescence emission from ≈400 nm (pyrene monomer, with a fluorescence lifetime of ≈5 ns) to 485 nm (pyrene excimer, with a lifetime of ≈40 ns) upon PDGF binding (Fig. 6C). Consequently, this design

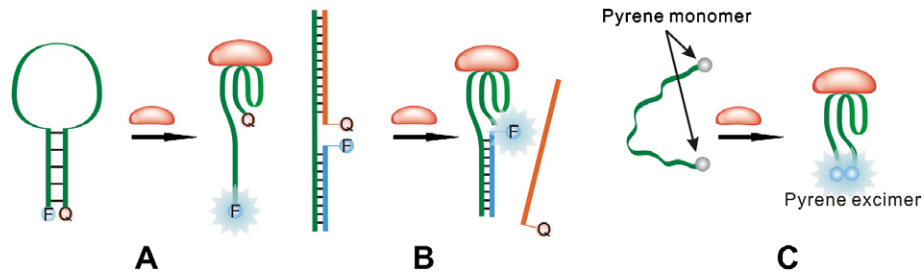

Figure 6. Aptabeacons. (A) An aptamer sequence in a molecular beacon-like hairpin structure was end-labeled with a fluorophore (F) and a quencher (Q). The binding of the target disrupted the stem, separating the F from the Q and leading to fluorescent signals [42]; (B) a fluorophore-labeled aptamer in a duplex structure with a complementary DNA sequence labeled with a quencher was separated after the binding of the target and the aptamer, leading to an increase in fluorescence [43,44]; and, (C) an aptamer labeled with one pyrene at each end switched its fluorescence emission and produced stable signals upon target binding [48].

circumvented the significant problem of background signal inherent in complex biological samples.

Much recent attention has been paid to the use of nanomaterials (e.g., AuNPs) that make it possible to develop simple colorimetric bioassay formats. In one of these approaches, unmodified AuNPs were used to discriminate unfolded and folded DNA; that provided a novel approach to probe aptamer-based bio-recognition processes colorimetrically (Fig. 7A). DNA aptamer is random-coil like in solution, which could tightly bind to AuNPs and stabilize them against salt-induced aggregation. Upon binding to its target, the aptamer formed 3D structures (e.g., G-quartet) and no longer protected AuNPs from being aggregated. Then, color changes of unmodified AuNPs are visible to the naked eye. To date, this assay strategy has been applied successfully to detection of potassium ion [49] and proteins [50].

Aptamers have also been employed recently to assemble NPs and carry out biosensing assays. In a related original study, aptamers were used as linkers to assemble ssDNA-functionalized AuNPs [51,52]. In the presence of target molecules, the aptamer switched its structure and the NPs dissociated (Fig. 7B). As a result, purple-colored aggregates separated into red-colored individual NPs. This assay format can be used to analyze a broad range of molecules by simple replacement of the aptamer sequences. It has been used to detect adenosine and cocaine in serum by a simple ''dipstick'' test [53].

More recently, the aptamer-linked AuNP system has been systematically investigated and compared with aptamer-beacon systems [54]. In another assay design [55], a DNA aptamer was first hybridized with a short complementary ssDNA self-assembled on AuNPs, which were well dispersed in solution and looked red. Upon binding of the target, the aptamer strands underwent a structure-switching event that led to their dissociation from AuNPs. The unhybridized AuNPs were unstable at the same salt concentration and aggregated immediately, leading to a rapid, red-to-purple color change (Fig. 7C).

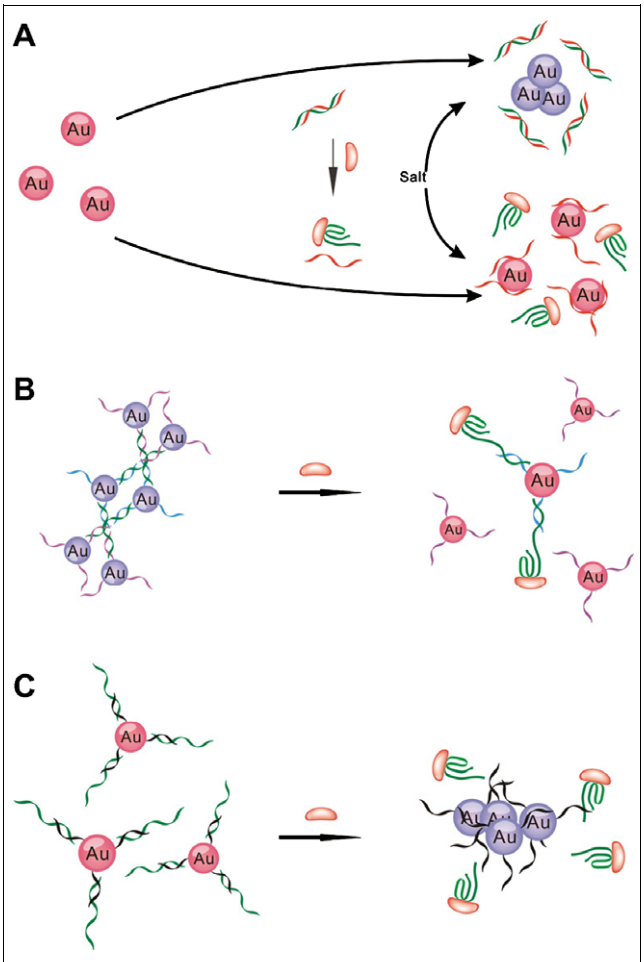

Figure 7. DNA aptamer could tightly bind to AuNPs and stabilize them against salt-induced aggregation. (A) Upon binding to its target, the aptamer formed 3D structures and led to the aggregation of AuNPs [49,50]; (B) in the presence of target molecules, purple-colored DNA-modified AuNP aggregates were dispersed into red-colored individual AuNPs [51,52]; and, (C) upon binding the target, AuNPs functionalized with short, complementary ssDNA and aptamers were unstable against salt-induced aggregate, leading to a red-to-purple color change [55].

Functionalized AuNPs with aptamers have also been used for the amplified optical detection of proteins in the sandwich configuration [56]. The anti-thrombin aptamer was covalently attached to a maleimide-functionalized siloxane monolayer, and thrombin was bound to the interface. The same aptamer-functionalized AuNPs were then associated to the second thrombin binding site. The resulting AuNP interface was then enlarged in growth solution containing $HAuCl_4$ and reducing agents (Fig. 8A). The enlargement of AuNPs significantly improved the colorimetric detection sensitivity, which could detect as few as 2 nM thrombin.

More recently, Li et al. reported a similar approach that employed silver amplification [57] (Fig. 8B). Their assay simultaneously employed an AuNP-aptamer and a biotin-aptamer that bound to the PDGF-BB, with an extremely low LOD of 83 aM.

4.3. Mass-sensitive

A mass-sensitive biosensor is defined as any device that measures the property that scales proportionally to mass associated with or bound to its sensitive surface assembled with capture probes. Aptamer-based, mass-sensitive biosensors are a class of label-free bioassays, including evanescent wave-based sensors (e.g., surface-plasma resonance (SPR), acoustic wave-based sensors (e.g., quartz crystal microbalance (QCM) and surface acoustic wave (SAW) devices), and micromechanical cantilever-based sensors). They are often referred to as ''mass-sensitive'' techniques because of the gravity or thickness measurements for thin films.

SPR sensors are devices capable of registering mass changes by the associated change in refractive index at the surface. Because SPR can determine the binding constants of aptamers and their targets, this technology is often used in SELEX process and performed robustly, precisely and rapidly. The SPR method is also applied for aptamer-based sensing applications. In this sensing format, a selective surface is formed by immobilizing the aptamer on the surface. The target is then injected at a constant flow rate, while the instrument measures

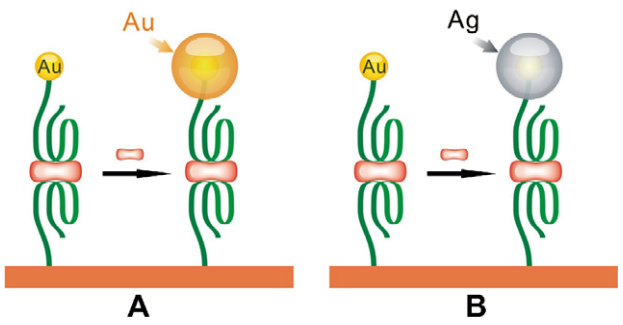

Figure 8. The ''sandwich'' type aptamer-based colorimetric bioassays with gold (A) [56] and silver (B) [57] amplification systems.

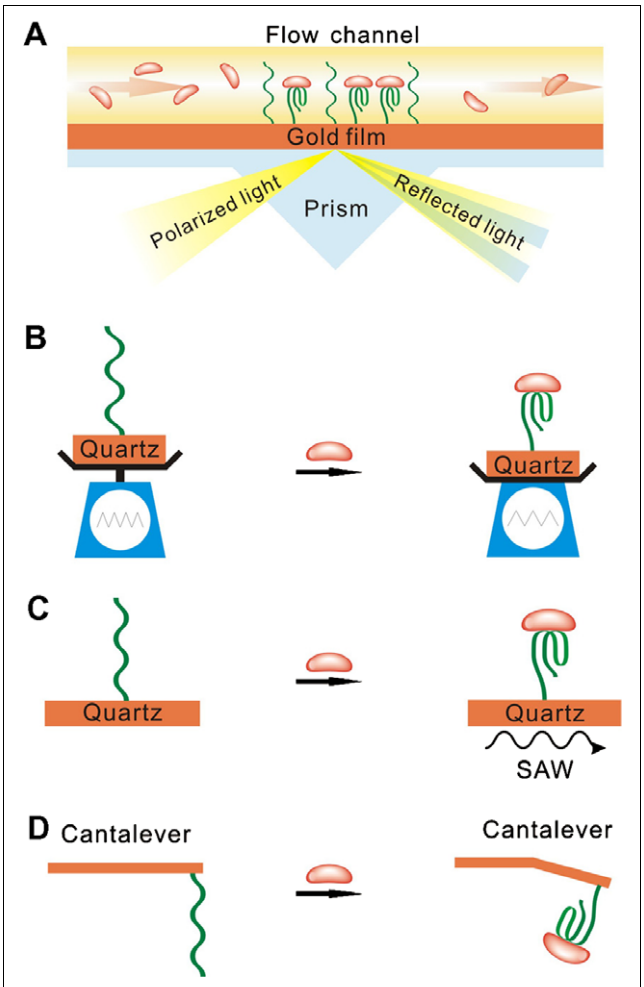

Figure 9. Mass-sensitive aptasensors. (A) SPR-based aptasensor [58,59]; (B) QCM-based aptasensor [59,61,62]; (C) SAW-based aptasensor [63]; and, (D) micromechanical cantilever-based aptasensor [65,66].

changes in the resonance angle that occur at the surface. The angle varies when the aptamer binds to the target (Fig. 9A). It was found that the signal was proportional to the bound molecules [58], thus allowing label-free detection in a single-site binding configuration. Recently, the aptamer specific for HIV-1 Tat protein was immobilized on SPR chips by using the avidin-biotin bridge [59]. The high assay specificity was identified by using a control protein Rev that was similar to Tat. Using the two-site binding model, the signal-amplification system can be involved to develop highly sensitive, highly specific SPR bioassays.

More recently, an enzymatically-amplified SPR imaging (SPRI) assay was developed to detect thrombin and vascular endothelial growth factor (VEGF) [60]. First, the target proteins bound to the immobilized aptamer on the surface, and then a horseradish peroxidase (HRP)-conjugated antibody was introduced to create an aptamer-target-antibody sandwich structure. This surface was then exposed to the HRP substrate that formed a dark

blue precipitate on the surface. A very small amount of this precipitate on the Au surface resulted in a significantly amplified SPRI response and the high sensitivity was achieved (LODs for thrombin and VEGF were 500 fM and 1 pM, respectively).

Micro gravimetric analysis on QCM has been used to detect interaction of aptamers and their targets. The frequency of the quartz crystal is controlled by changes in the mass associated with the crystal, thus the association of a target onto aptamer-modified crystals increases the mass on the transducer, resulting in a decrease in the resonance frequency of the crystal (Fig. 9B).

Recently, two similar methods used a bintinylated aptamer immobilized on the Au/quartz crystal for the microgravimetric sensing assay of thrombin and HIV-1 Tat protein with LODs of 1 nM and 0.25 ppm, respectively [59,61].

The parameters of QCM-based aptasensors have also been evaluated and optimized to improve their analytical performances [62]. Besides the applications in QCMs, piezoelectric quartz crystals are also used to fabricate SAW-type aptasensors. A special type of SAW sensor, the Love-wave sensor, has been developed to detect multifunctional serine protease thrombin and Rev peptide [63] (Fig. 9C). Its LODs were approximately 72 pg/cm^2 and 77 pg/cm^2 for thrombin and Rev peptide, respectively. More recently, a Love-wave aptasensor array based on this sensor system was developed for monitoring complex formation in the blood-coagulation cascade [64].

Micromachined biosensors have low noise and high scalability due to their small size. The low noise results in higher resolution, while scalability allows many sensors to be used in parallel for point-of-care systems. In such an aptamer-based assay, the aptamers are bound to the top surface of the microcantilever. Aptamers binding with their targets lead to steric crowding that forces the cantilever to bend. The bending can thus be detected optically or electronically (Fig. 9D).

Using a microfabricated cantilever-based sensor that was functionalized with DNA aptamers, Savran et al. reported a label-free detection sensor for Taq DNA polymerase [65]. The sensor utilized two adjacent cantilevers for direct detection of the differential bending between them. One cantilever was functionalized with aptamers selected for, while the other was blocked with ssDNA. The polymerase-aptamer binding induced a change in surface stress that caused a differential cantilever bending in the range 3–32 nm, depending on ligand concentration.

More recently, the nanomechanical microcantilevers operated in vibration mode (oscillation) using RNA aptamers as receptor molecules were fabricated for label-free detection of hepatitis C virus (HCV) helicase. Detection could be achieved at concentrations as low as 100 pg/ml HCV helicase [66].

5. Conclusion and trends

This review has presented an overview on recent advances in the development and the application of aptamer-based sensors. While such aptasensors emerged only about 10 years ago, they have already found broad applications in both basic research and biomedical diagnostics. A range of transducers (e.g., electrochemical, optical and mass-sensitive) have been employed in aptasensors. In particular, label-free sensing formats (e.g., SPR, QCM, SAW and micromechanical cantilevers) offer the promise of reagentless, one-step analyses.

In spite of these rapid advances, aptamer-based bioassays are still immature when compared to immunoassays, which in a sense reflects the limited availability of aptamer types and the relatively poor knowledge of surface-immobilization technologies for aptamers.

Nanotechnology has made an important addition to the analytical and diagnostics workbox [67] (e.g., the combination of aptamers with novel nanomaterials has led to highly sensitive and selective aptasensors).

In short, while there is still a long way to go, we expect that aptamer-based biosensors will eventually become a real-world tool that could meet challenges that would otherwise be impossible with currently available technologies.

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