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REVIEW

Aptamer-based molecular recognition for biosensor development

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Abstract Nucleic acid aptamers are an emerging class of synthetic ligands and have recently attracted significant attention in numerous fields. One is in biosensor development. In principle, nucleic acid aptamers can be discovered to recognize any molecule of interest with high affinity and specificity. In addition, unlike most ligands evolved in nature, synthetic nucleic acid aptamers are usually tolerant of harsh chemical, physical, and biological conditions. These distinguished characteristics make aptamers attractive molecular recognition ligands for biosensing applications. This review first concisely introduces methods for aptamer discovery including upstream selection and downstream truncation, then discusses aptamer-based biosensor development from the viewpoint of signal production.

Keywords Molecular recognition · Biosensor · Ultrasensitive detection · Nucleic acid aptamer · Upstream aptamer selection · Downstream aptamer truncation

Introduction

Molecular recognition plays an essential role in nature. Various biomolecular pairs can be easily observed in biological systems, including antibodies and antigens [1], hormones and hormone receptors [2], DNA promoters and transcription factors [3], enzymes and substrates [4], etc. Most have high binding strength and specificity and their interactions are well studied. These naturally occurring biomolecular pairs are of great interest for various biological and biomedical applications, one of which is biosensing. A variety of biosensors have been explored since the development of the first enzyme-based biosensor over 40 years ago [5]. However, these biomolecular systems have not evolved in nature purposely for the uses the human being expects. For instance, many biomolecules (e.g., enzymes and antibodies) have fragile functional structures. When placed in an environment that is different from their ideal conditions they can be easily denatured and lose their initial functionality. Therefore, these molecular pairs have limited analytical applications in many situations. Clearly, it is of great interest and importance to explore synthetic ligands that not only recognize target molecules, but also have special characteristics such as tolerance of harsh chemical and biological conditions.

Nucleic acid aptamers are one type of synthetic ligands that hold great potential for various biosensing applications. Aptamers are single-stranded nucleic acid ligands that are selected to have high binding affinity and specificity to target molecules [6, 7]. Nucleic acid aptamers have been recognized as "chemical antibodies" because they interact with their targets with high binding affinity and specificity comparable with the interactions between antibodies and antigens. Additionally, synthetic aptamers have unique characteristics not found in natural antibodies. First, aptamers can be chemically synthesized in an easy and reproducible manner. Second, nucleic acid aptamers can be selected in either physiological or non-physiological conditions against a variety of target molecules [8–13]. Third, chemically modified aptamers can have superb stability in harsh conditions [10]. Fourth, the formation of aptamer-target complexes can be reversed in an active manner via

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intermolecular hybridization under physiological conditions [14]. Because of these distinguishing features, aptamers have been explored for various biological and biomedical applications, for example cancer therapy [15], drug delivery [16], and biosensor development [17–26].

Several excellent and comprehensive reviews describing the development of biosensors have already been published [27–36]. Thus, this review offers an introduction on the use of aptamers for biosensor development by highlighting the most recent research activities on aptamer discovery then discusses aptamer-based biosensor developments from the viewpoint of signal production.

Nucleic acid aptamers

Nucleic acid aptamers have been selected to bind to a broad range of targets including small inorganic and organic molecules (K^+, Hg^{2+}, ATP, cocaine, etc.), large biomolecules (peptides and proteins), and whole organisms (bacteria and cells) [37–41]. The discovery of nucleic acid aptamers involves two critical steps. The first is the upstream selection, *i.e.*, the discovery of full-length aptamers from single-stranded DNA or RNA libraries with a process named Systematic Evolution of Ligands by Exponential Enrichment (SELEX) [6, 7]. The second is the downstream truncation, *i.e.*, the identification of the essential and nonessential nucleotides of the full-length aptamers [42].

Aptamer selection

Figure 1 shows a typical SELEX process. The process begins with the generation of a random library that contains up to 10^{14}–10^{15} single-stranded DNA or RNA oligonucleotides. The oligonucleotides are synthesized by a combinatorial chemical synthesis technique. These oligonucleotides are comprised of *N* random nucleotides in the middle of their sequences, flanked by a primer-binding region at each end. During the selection of the aptamers, target molecules are incubated with the library to enable molecular recognition between the target molecules and the oligonucleotides in the library. Theoretically, the aptamers will tightly bind to the target molecules and the other oligonucleotides will stay in the binding solution. Because the aptamer–target complexes and the free oligonucleotides in the binding solution should have different physical properties, they can be separated with a partitioning method or tool. After separation, the sequences bound to the target molecules will be amplified to generate a new library for further selection. This process is usually repeated for 8–12 cycles.

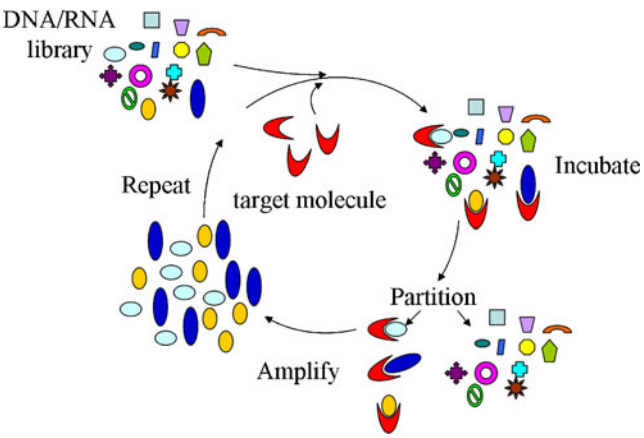

Fig. 1 The general procedure for SELEX. The initial library contains up to 10^{14}–10^{15} DNA or RNA oligonucleotides that are generated by combinatorial chemical synthesis. Identification of high affinity aptamers usually relies on many cycles of aptamer selection. Each selection cycle involves incubation, partition, and amplification

The most critical step of SELEX is the separation of bound oligonucleotides from the unbound oligonucleotides. Nitrocellulose filtration is the most commonly used technique [6, 7]. As aptamer-related research grew at an increasing pace within the past few years, numerous separation techniques have been used to improve aptamer selection, including affinity surfaces (e.g., affinity magnetic beads [43], titer plates [44], and affinity chromatography [45, 46]), gel mobility shift [47], immunoprecipitation [48], density-gradient centrifugation [49], and tailored SELEX [50]. These techniques are promising to improve aptamer selection. However, no systematic studies have been carried out to compare their effectiveness in improving aptamer selection. In addition to the development of separation techniques for improving the separation efficiency, other SELEX techniques such as photo-SELEX [51], evolution-mimicking algorithm-based SELEX [52], and molecular beacon-based SELEX [53] have been developed to improve the selectivity of the aptamer. To further advance the breadth of aptamer selection, cell-SELEX [41] and tissue-SELEX [54] have recently been studied. These methods have the potential to identify aptamers that can recognize cancer cells or tumor tissues for cancer therapy and diagnosis. However, the aptamer selection process is iterative. It can be very time-consuming, requiring several months or longer to complete. To overcome this problem, automated SELEX [55, 56] and microarray-based SELEX [57] have been developed. These new methods are promising to improve the speed of aptamer-based research.

Aptamer truncation

The aptamers identified by the selection procedure (i.e., full-length aptamers) are usually 80–100 nucleotides long. However, not all of the nucleotides in a full-length aptamer

play a critical role in binding to its target. A full-length aptamer usually has three functional regions. The region that plays the role of contacting the target is approximately 10–15 nt long [58]. Those nucleotides have secondary structures such as hairpin loops, G-quartet loops, bulges, or pseudoknots. Another region contains nucleotides that do not directly contact the target but play an important role in supporting the interactions between the contacting nucleotides and the target [58]. The nucleotides that either bind to the target or facilitate aptamer binding are the essential nucleotides required for a functional aptamer. In general, the number of essential nucleotides is approximately 25–40 nt [59]. The third region comprises the nucleotides that do not bind to the target nor support the binding of the contacting nucleotides to the target. They are regarded as nonessential nucleotides. It is always desirable to truncate the aptamer to eliminate the nonessential nucleotides after the upstream aptamer-selection process (i.e., downstream truncation). There are several reasons for doing the downstream truncation. First, in some cases, elimination of nonessential nucleotides can increase the binding functionality of the aptamer [42, 60] presumably because of reduced steric hindrance. Second, the smaller aptamers can provide more freedom and space for constructing novel nanostructures with smaller, more desirable dimensions, which is required to develop tiny, more sensitive biosensing devices [61]. Third, although the chemical synthesis of an oligonucleotide is a well-established technique, the synthesis of aptamers longer than 60 nt is always more expensive and difficult to perform.

Aptamer truncation is usually performed on the basis of analysis of simulated structures or characterization of aptamer segments. The former uses algorithms to theoretically predict the secondary structures of aptamers [62–67]. By comparing structural similarities between the selected aptamers, one would determine which segments of the aptamers are essential sequences for binding. For instance, this technique has been successfully used for the truncation of anti-hPTK7 aptamers [64]. However, current algorithms predict the structures of nucleic acids in the absence of target molecules whereas it is possible for some, if not all, aptamers to undergo conformational changes upon binding to the target molecules [68]. Therefore, this technique will have broader applications in directing aptamer truncation if the current algorithms can be further improved. The latter, which is more commonly used, is the massive synthesis and analysis of shorter sequences of an aptamer. The shorter sequences are usually generated by chemical synthesis, enzymatic fingerprinting, boundary analysis, or RNA transcription [43, 69–72]. The usefulness of these elegant approaches is compromised because they are often complicated, expensive, or time-consuming [71]. For instance, the cost of chemical synthesis of all the shorter sequences of a single full-length RNA aptamer would be astronomically high.

Recently, we developed a new method for aptamer truncation by using complementary oligonucleotides as molecular guides to map the functionality of the aptamers (Fig. 2) [42]. The concept of this method is twofold. First, when complementary oligonucleotides hybridize with the nonessential nucleotides of an aptamer, the interaction between the aptamer and its target is not significantly interfered with. Second, when complementary oligonucleotides hybridize with the essential nucleotides, the interaction can be interfered with and the degree of interference is dependent upon the number of hybridized essential nucleotides. On the basis of this method, aptamer truncation can be completed in less than a week.

Nucleic acid aptamer-based biosensors

Signal production without the aid of special molecular reporters

The essential role of a biosensor is to capture analytes and produce a detectable signal. During the process of analyte capture, the physical properties (e.g., mass and conductivity) on the surface of a biosensor will change. On the basis of these principles, aptamers have been used to develop mass-responsive or electrochemical biosensors.

Aptamers have been conjugated with biochips for the development of surface plasmon resonance (SPR) sensors. SPR is a method for measuring changes in the refractive index of a sensor surface [73]. When analytes in a solution bind to the aptamers immobilized on the biochip surface, the overall mass on the biosensor surface is going to change. As a result, the refractive index close to the sensor surface will change. This change is the SPR signal. The SPR signal is proportional to the amount of bound molecules. Thus, by measuring the change of the signal, one can determine the total amount of analytes that exist in

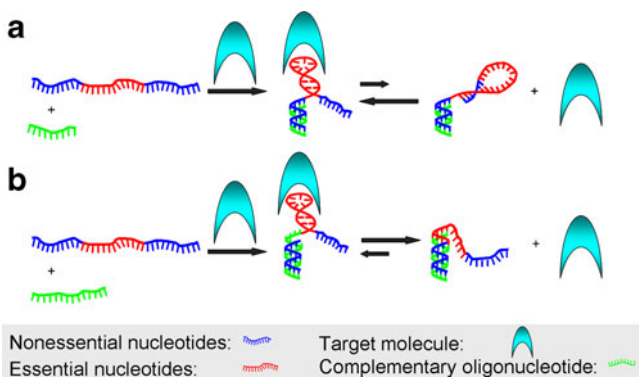

Fig. 2 Aptamer truncation based on intermolecular hybridization. (**a**) The nonessential nucleotides of an aptamer are hybridized with a complementary oligonucleotide. (**b**) The essential nucleotides of the aptamer are hybridized

the running solution. Aptamer-based SPR analysis has been used to detect the C-reactive protein [74], the HIV-1 Tat protein [75], human IgE [76, 77], and retinol binding protein 4 (RBP4) [78]. Promising results have been obtained. For instance, in a study pursued by Lee et al. [78], a DNA aptamer was selected to specifically bind to RBP4, a useful biomarker for monitoring early type 2 diabetes. The immobilized RBP4 aptamer on the SPR chip could be used to detect low levels of RBP4 in serum samples. The aptamer-based SPR biosensing had a linear range between 0.2 and 0.5 $\mu g mL^{-1}$, which was slightly more sensitive than the competitive enzyme-linked immunosorbent assay (ELISA) that had a linear range between 0.3 and 0.7 $\mu g mL^{-1}$.

Aptamers have also been coated on the surface of quartz crystals to develop quartz crystal microbalances (QCM) [79]. A QCM is an acoustic wave resonator. Because of the piezoelectric nature of quartz material, application of an alternating electric field can lead to the generation of acoustic waves that propagate through the bulk of the material. When analytes are captured on the surface of the quartz crystal, the mass will increase. The mass increase will lead to resonant frequency variation. On the basis of the Sauerbrey equation that establishes a linear relationship between the mass increase and the frequency variation, the concentration of analytes in the solution can be measured. Recently, an aptamer-based QCM has been investigated for detection of the HIV-1 Tat protein [75] and thrombin [80]. The limits of detection (LODs) for the HIV-1 Tat protein and thrombin were 0.25 ppm and ~1 $nmol L^{-1}$, respectively. An aptamer-based QCM has also been used for the detection of IgE. The LOD was 2.5 $\mu g L^{-1}$. In addition, a linear detection range was observed when the concentration of IgE was varied between 2.5 $\mu g L^{-1}$ to 200 $\mu g L^{-1}$ [81]. Similar to QCM, a love mode surface acoustic wave (SAW) technique could also be applied for biosensing devices utilizing nucleic acid aptamers. For instance, a love wave SAW biosensor has been used for thrombin detection with an LOD of ~72±11 $pg cm^{-2}$. Studies have also been carried out to combine SAW-based biosensing with other detection devices for miniaturized diagnostics. For example, Treitz et al. developed a SAW–MS device that could capture and directly identify the target by means of an aptamer-modified SAW and matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry [82].

Clearly, previous studies demonstrated that high sensitivity and low LODs could be achieved by using aptamer-based SPR, QCM, and SAW. However, these elegant systems may not be applicable to the simultaneous detection of multiple analytes. To achieve such an objective, microfabrication techniques have been studied to improve the scalability of biosensors. A typical example is the microfabricated cantilever-based biosensor [24]. The binding of different target proteins is detected by measuring the differential cantilever bending between the sensor cantilever (the aptamer-modified surface) and the reference cantilever (the single-stranded control DNA-modified surface). The differential cantilever bending could range from 3 to 32 nm, which is detectable by use of an interferometer.

Different from the mass-dependent measurement devices, several label-free electrochemical biosensors were developed based on changes in conductivity because of aptamer–target interactions [83, 84]. The electron-transfer resistance of the conducting support will change on interaction between the aptamer and its target molecule. Based on this principle, Radi et al. explored a reusable biosensor for thrombin detection [84]. After the binding of thrombin, the changes in resistance were probed in the presence of $Fe(CN)_6^{3-/4-}$ and analyzed by electrochemical impedance spectroscopy. Because the thrombin aptamer was directly immobilized on the sensor surface without other modifications, this biosensor could be regenerated by removing the bound thrombin from the biosensor surface after analysis. Aptamers have also been integrated with many novel nanomaterials for biosensor construction, because of their small size and high sensitivity. One representative application is the carbon nanotube field-effect transistor (CNT-FET). When compared with antibodies, aptamers are more suitable for CNT-FET biosensors. Because antibodies are much larger in size than the Debye length (the most significant charge-separation distance), antibody-based recognition tends to occur outside the electrical double layer in solution, resulting in a low sensitivity. On the other hand, the smaller size of aptamers (1–3 nm) will guarantee that protein recognition occurs within the Debye length. It turned out that the sensitivity of an aptamer-based CNT-FET is much higher (e.g. a LOD of 250 $pmol L^{-1}$ for IgE detection) than that of the antibody-based one [61].

The aptamer-based biosensors described above are based on changes in mass or conductivity. However, it is a challenging task to apply these promising methods to the detection of small molecules such as ATP and cocaine, because the signal change resulting from binding of small molecules is usually undetectable. To achieve more sensitive analysis, utilization of molecular reporters has been studied.

Signal production with the aid of molecular reporters

The current trend in analytical and bioanalytical chemistry is to develop novel technology that does not rely on expensive or complicated instruments. In addition, this technology should be able to detect a large array of analytes, from large entities (e.g. proteins and cells) to small molecular weight species (e.g. ATP). Nucleic acid

aptamers functionalized with molecular reporters have the potential to achieve these objectives. Because nucleic acid aptamers are single-stranded DNA or RNA molecules, their inherent flexibility allows them to fold into specific tertiary structures upon binding to their target molecules. In addition, their structures can be controlled or modulated by intermolecular hybridization when they make contact with their shorter complementary sequences. These features have been useful for incorporating molecular reporters for the developing of biosensors and probes.

Optical reporters have been chemically conjugated to aptamers for detection of analytes [85]. A common type of probe is the molecular beacon. In a molecular beacon, a fluorophore and a quencher are attached to the 3′ and 5′ ends of the aptamer. In the absence of analytes, some aptamers have a random, flexible format. In the presence of analytes, the aptamer and the analyte will form a compact nanostructure so that the 3′ and 5′ ends of the aptamer will be brought close to each other. Thus, the fluorescent signal can be turned from an "on" to an "off" state (Fig. 3a). This concept has been applied to the development of an anti-cocaine probe [86], an anti-thrombin probe [87], and an anti-PDGF-BB probe [88]. On the other hand, other aptamers initially have a well-defined stem structure because of the intramolecular hybridization of the 3′ and 5′ end regions. When the aptamer binds to an analyte, the stem region has a chance of splitting apart. In another words, the hybridized nucleotides in the stem region can be dissociated in the presence of analytes because of the analyte-induced conformational change of the aptamer [89]. Thus, the structural change has a capability of inducing the separation of the quencher from the fluorophore to a certain distance. The fluorescent signal can be turned from an "off" to an "on" state. On the basis of this principle, fluorescence resonance energy transfer (FRET) probes have been studied [90].

Analyte-induced structural changes of single aptamers have also been combined with electrodes for biosensor development. Redox-active methylene blue (MB) [91] is one of the most commonly used reporters. For instance, an anti-thrombin aptamer bearing MB was studied for the detection of thrombin in blood serum [92]. Upon binding to thrombin, the aptamer folded into a G-quadruplex conformation and the MB molecules were shielded from communicating with the electrodes by electron transfer. The signal changed from an "on" to an "off" state [92]. Similarly, a MB-modified anti-cocaine aptamer was recently investigated for real-time monitoring of cocaine in blood serum [25, 93]. On the other hand, researchers found that

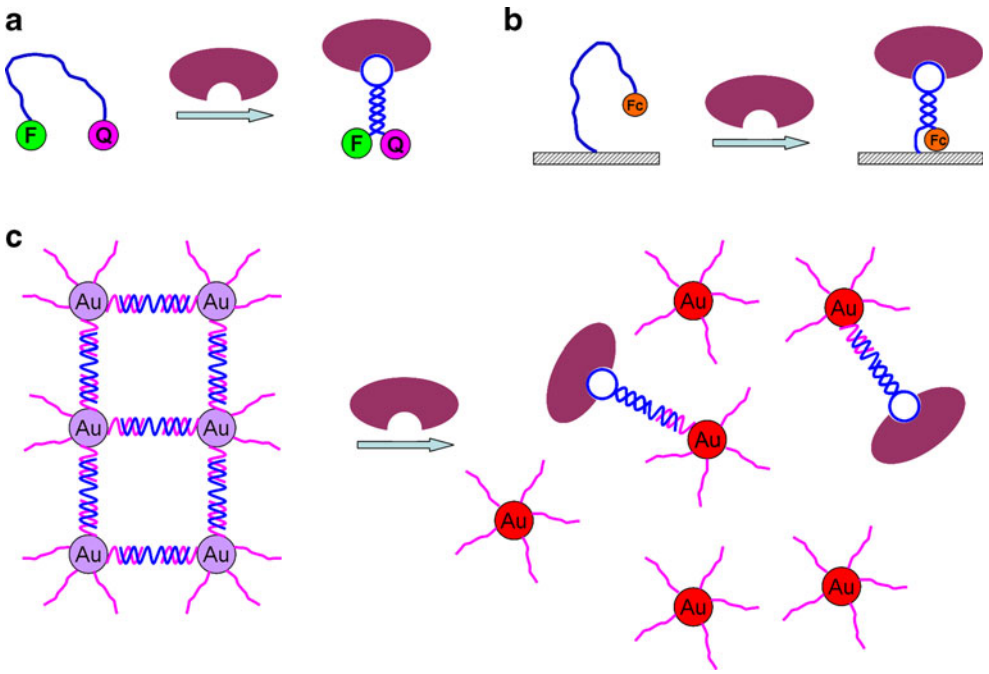

Fig. 3 Signal production with the aid of reporters. (**a**) An aptamer-based molecular beacon system. An aptamer is end-labeled with a fluorophore (*F*) and a quencher (*Q*). The binding of the target molecule will induce the structural change of the aptamer, bringing *F* and *Q* together to "turn off" the fluorescent signal. (**b**) An electrochemical biosensor employing an aptamer carrying a redox-active molecule at its end. The binding of the target protein will force the end-labeled ferrocene (*Fc*) toward the electrode to provide continuous electronic communication of the ferrocene with the electrode. (**c**) An intermolecular hybridization-mediated molecular detection. The aptamer is used as a cross-linker to assemble AuNPs together to form aggregates. The binding of the target molecule will lead to dissembling of the aggregates and cause the color change from purple to red

the binding of a target analyte could result in the formation of a G-quadruplex conformation and force the end-labeled reporter (e.g., ferrocene) toward the electrode. Therefore, when analytes are introduced to the system, the signal can be turned from an "off" to an "on" state (Fig. 3b) [94–96].

Clearly, the success of the single aptamer-based strategy is dependent on whether enough distance can be created between the molecular reporter and its quencher or the surface of an electrode. The rational design of the aptamers is a critical step for developing this type of aptamer-based biosensor or probe. Intermolecular hybridization can generate a rigid double-stranded structure that can create the desired distance between the two ends of an aptamer. In addition, intermolecular hybridization can interfere with the formation of functional structures of the aptamers whereas target analytes can compete with the complementary oligonucleotides to regenerate the functional structures of the aptamers. Therefore, the principle of intermolecular hybridization has also been studied for biosensor development.

The principle ideas mentioned above have been extended to create an aptamer-based biosensor in which intermolecular hybridization occurs with an immobilized aptamer on the surface of an electrode. Here, one end of the aptamer is directly conjugated to the electrode while the other is conjugated with a reporter. The formation of a rigid double-stranded structure is used to push the reporters away from the surface of the electrode. During the analysis, the presence of analytes induces the formation of aptamer–analyte complexes where the complementary oligonucleotide is released and the signal tag is brought close to the surface of the electrode [97]. In addition to organic reporters, inorganic nanoparticles have also been used for biosensing. A metal nanoparticle can be regarded as a large molecular reporter. One promising metal reporter is the gold nanoparticle (AuNP). In a state of aggregation, AuNPs are purple in color whereas in a state of dissipation they are red. The state transformation between aggregation and dissipation, and thus the color transformation of AuNPs has been used to develop aptamer-based biosensors and probes (Fig. 3c). For instance, aptamers have been used as cross-linkers to assemble complementary oligonucleotide-functionalized AuNPs to form aggregates [98]. When the target molecule was introduced, the aptamers refolded into a defined structure and released the complementary oligonucleotide-functionalized AuNPs. Therefore, the color of the system changed from purple to red, which provided a detectable optical signal [99, 100]. Studies have also been carried out to stabilize complementary oligonucleotide-functionalized AuNPs through partial intermolecular hybridization with nucleic acid aptamers. The presence of analytes induced the dissociation of aptamers from the AuNPs, which triggered the aggregation of AuNPs. This resulted in the color change from red to purple [101, 102]. By using these AuNP reporters, an LOD for thrombin as low as 0.83 nmol L^{-1} was reached [101].

Intermolecular hybridization-mediated signal production can also be performed in a three-oligonucleotide system. The system includes an aptamer and two complementary oligonucleotides. Both complementary oligonucleotides can partially hybridize with the aptamer. One complementary oligonucleotide carries a fluorophore and the other carries a quencher. When both of the complementary oligonucleotides hybridize with the aptamer, the fluorophore and the quencher are brought close to each other so that the fluorescent signal can be turned off. When target analytes are introduced to the system, the binding of the analyte to the aptamer will induce structural changes of the hybridized aptamer and the complementary oligonucleotide carrying the quencher will be released from the aptamer. Thus, a fluorescent signal will be produced [103]. To further reduce the interference of background signals in complicated samples, a wavelength-shifting method has also been studied [104, 105].

Intermolecular hybridization is a useful strategy for development of aptamer-based biosensors. However, it is important to control the hybridization length and the region between the aptamer and the complementary oligonucleotide. For instance, if the hybridization length is too long, the hybridized duplex may be too stable to be disrupted, even in the presence of target molecules [42]. On the other hand, if the hybridization length is too short, the release of the complementary oligonucleotide from the hybridized aptamer may easily generate background noise.

Signal production through an amplification mechanism

It is often necessary to detect some analytes with very low concentration in a sample. However, direct measurement of the analytes may not be feasible because many analytical tools have their inherent limitation of detection. Therefore, strategies have been explored to amplify the concentration of an analyte indirectly for ultrasensitive analysis that can lead to the detection of an analyte at an attomolar level [106]. Simply speaking, an analyte is represented by a large number of identical reporters. Therefore, by analyzing the concentration of the reporters, one can determine the concentration of the analyte in the sample. Aptamers have been used as a key element to develop various amplification-dependent biosensing systems (Fig. 4). For instance, aptamers have been used to functionalize plate surfaces, enzymes, nanoparticles, and DNA amplifiers.

One system is virtually the same as the conventional ELISA setup. In this application, the aptamer-functionalized plate was applied in a sandwich aptamer-linked immobilized sorbent assay, which was analogous to the signal

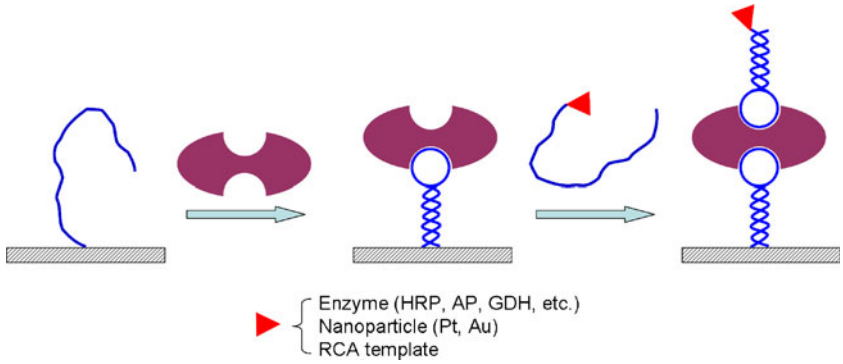

Fig. 4 An amplification-dependent sandwich-like biosensing system

amplification-dependent ELISA assay. In some studies, aptamers and antibodies have been used together in the same system. During the analysis, target proteins are first captured by capture aptamers immobilized on the solid surface. After removing the nonbinding proteins, enzyme-linked aptamers or enzyme-linked antibodies are added to the system. This enzyme-linked "sandwich" will then be exposed to enzyme substrates for signal amplification [23, 107–112]. Recently, aptamer-tethered Pt nanoparticles were proposed as substitutes for the enzymes to catalyze the reaction. As a result, the enzymatic reaction rate of Pt-catalyzed reaction was found to be one to two orders of magnitude higher than that of horseradish peroxidase (HRP)-catalyzed one [113]. In these aforementioned assays, aptamers are used as linkers rather than signals. In fact, studies have been carried out to use immobilized aptamers as signals for biosensing [114]. Hesselberth et al. has used multifunctional RNA aptamers (i.e., aptazymes) to detect diverse analytes [114]. In this method, the binding of analytes to aptamers triggers the allosteric activation of RNA nanostructures. The activated aptazymes react with the biotinylated substrate, which leads to their immobilization to a streptavidin-coated plate surface. Because the aptazymes are radiolabeled, detection of the radioactivity enables the measurement of the analytes.

Different from the biosensing methods that need immobilization of aptamers on the surface and multistep separation processes, signals can be directly produced in a homogeneous solution by using bi-specific aptamers [115]. The bi-specific aptamers are composed of two aptamer units. One unit is an analyte-binding aptamer; the other is an enzyme-binding aptamer. In the presence of analytes, bi-specific aptamers are subjected to structural changes. The structural changes lead to the activation or inactivation of the enzyme-binding aptamer, dependent on the design of the bi-specific aptamers. As a result, signals can be produced on the basis of enzyme activity [115].

Another system utilizes aptamer-functionalized nanoparticles as a support for reporter molecules. For instance, AuNPs have been used to carry a large number of aptamers with a 25-adenine tail [111]. After forming the complexes with the captured target proteins, the poly-adenine tails are hydrolyzed by a nuclease to release a large number of adenines as the signal molecules to be detected. The LOD can reach 0.1 $ng\,mL^{-1}$ for thrombin analysis by using this method [111]. Similarly, aptamer-functionalized AuNPs can be used to carry smaller CdS nanoparticles [116]. Because each CdS nanoparticle contains many Cd^{2+} ions, the signal can be significantly amplified after the release of Cd^{2+} ions under acidic conditions. The released Cd^{2+} ions can be easily detected by differential pulse voltammetry (DPV). This ultrasensitive biosensor could detect as little as 0.55 $fmol\,L^{-1}$ thrombin [116]. Aptamer-functionalized AuNPs can also be used as a support to attract Au^{3+} or Ag^{+} ions for detection instead of releasing any reporter molecules from its surface. This method is based on densitometric detection through an enhanced aggregation process. The Au^{3+} or Ag^{+} ions in an enhancer solution will aggregate on the surface of the initial AuNPs to form larger metal particles for signal amplification and detection. This method has been successfully adapted for ultrasensitive detection of thrombin [117] and PDGF-BB [118].

Aptamers can also be used by themselves [119] or carry a specific DNA tail [120] for detection of analytes on the basis of polymerase chain reaction (PCR). The use of PCR can amplify a few copies of DNA by several orders of magnitude to generate thousands or millions of copies [121]. Thus, after forming complexes with target proteins, the aptamers or their tails can be amplified through PCR to acquire strong signals. This method can be further improved by using real-time rolling circle amplification (RCA) [122–125]. RCA have several advantages over conventional PCR. Conventional PCR analysis relies on a thermocycler for cyclic reactions. In contrast, RCA is an isothermal method. It can be carried out at body temperature. The elongated RCA product with thousands of nucleotides can be detected by various approaches, for example fluorescent probes [126] or oligonucleotide-tethered particles [125].

Conclusion and outlook

Nucleic acid aptamers are an emerging class of synthetic affinity ligands. They can recognize a large range of molecules with high binding affinity and specificity. They have numerous merits for biosensing applications in comparison with most affinity ligands evolved in nature. This review concisely describes the methodology used for aptamer discovery. In particular, this review covers recent research activity in the development of aptamer-based or aptamer-related biosensing systems. From the information presented in this review it is clear that significant progress has been made in applying nucleic acid aptamers for detection. However, some challenges still remain. First, despite twenty years of effort, the total number of nucleic acid aptamers discovered so far is still much less than that of antibodies. Various elegant methods for aptamer selection and truncation have recently been developed. Unfortunately, many of these (e.g., microfluidic aptamer selection [127]) need specific expertise and have not been widely used by many laboratories. Thus, aptamer discovery is still a trial-and-error procedure in most laboratories. Because nucleic acid aptamers play the essential role in developing aptamer-based biosensing systems, it is highly desirable to create new, yet easy and universal methodology for aptamer discovery. Second, understanding of molecular recognition between aptamers and target molecules lags behind the development of new biosensing technology. For instance, many aptamer-based biosensors depend on intermolecular hybridization. These biosensing systems involve at least three components: aptamers, complementary oligonucleotides, and analytes. However, the molecular recognition in such a multi-component system is still not well understood. Third, although aptamers are selected for high affinity and specificity, a biosensing surface will not be completely covered by aptamers. Nonspecific adsorption of molecules by the areas without aptamers can be problematic in terms of the improvement of sensitivity. In addition, the high affinity and specificity may be changed under some conditions (e.g., high salt concentration). In particular, most biosensing systems have been developed for clinical applications. The human samples contain various small and large molecules with varied concentrations in different patients. The working environment is different from a defined selection buffer. It may exaggerate the issue of nonspecificity. Fourth, many elegant aptamer-based biosensing systems are still not highly sensitive in comparison with conventional ELISA. However, it is important to note that aptamer-based research and biosensing applications are still in their infancy in comparison with many well-established bioanalytical tools. It is believed that the efforts of biologists, chemists, and engineers will eventually lead to the successful development of numerous aptamer-based biosensing systems.

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