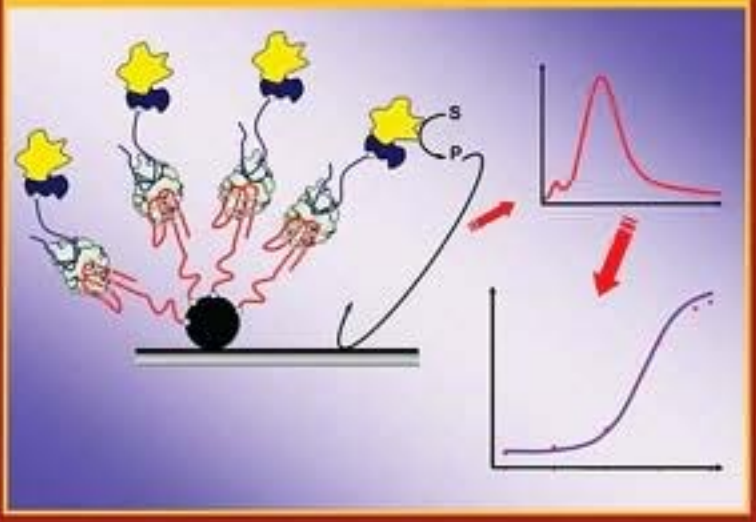
Aptamers in Bioanalysis



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CHAPTER 12

STRATEGY FOR USE OF SMART ROUTES TO PREPARE LABEL-FREE APTASENSORS FOR BIOASSAY USING DIFFERENT TECHNIQUES

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12.1 INTRODUCTION

Biosensors, which usually need biological molecules as recognition elements, are playing a growing role in various fields, due to their effective selectivity, high sensitivity, and easy performance. Thus, even for only one useful target, it is worth large numbers of designs and improvements to develop detection that is effective enough for a variety of applications. In this way, more and more techniques and novel recognition elements are being brought into bioassays.

Aptamers are screened through the systematic evolution of ligands by exponential enrichment (SELEX) process as functional oligonucleotides (Ellington and Szostak, 1990; Robertson and Joyce, 1990; Tuerk and Gold, 1990). Because of their generally impressive selectivity and affinity, they have already attracted much analyst attention and were quick to be used in analytical assays as novel recognition elements. Aptamers exhibit multifarious advantages over traditional recognition elements (Osborne and Ellington, 1997; Jayasena, 1999; Famulok et al., 2000; Tombelli et al., 2005a). For example, they are easy to synthesize in vitro, easy to modify, and flexible to design. These inherent advantages as oligonucleotides not only make target diversification but also improve detection efficiency. They can also help analysts simplify the analytical process and fabricate a sensing platform smartly and conveniently.

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Until now, a large number of aptasensors developed in the past were rationally designed. Several of them have been proven feasible in practical biosamples. Various techniques to get a detectable signal have been used or combined, such as optical transduction (Lee and Walt, 2000), quartz crystal microbalance (QCM) (Liss at al., 2002), surface plasmon resonance (SPR) (Tombelli et al., 2005b), fluorescence (Nutiu et al., 2004; Pavlov et al., 2005; Yang et al., 2005; Rupcich et al., 2006), colorimetry (Lu and Liu, 2006, 2007), chromatography (Ravelet et al., 2006), and electrochemistry (Willner and Zayats, 2007). On the whole, among all the convenience-aimed aptasensors, label-free seems to be one of the most widely used methods with whatever detection techniques are adopted.

Label-free was at first employed to describe a process of avoiding a label (or rather, a radioactive label) on analytes [the analyte label-free (ALF) mode] and has attracted more and more attention since it appeared. The term has become a synonym for techniques that can release any one operation from labeling and is now considered to be one of the most promising areas in analytical science. In addition, such a label-free method is useful not only in basic research in laboratories but also exhibits significant market potential and promise in practical applications.

To some degree, the advantages of label-free detection are remarkable and are summarized in Table 12.1. As shown in the table, label-free is one of the most effective methods to make detection simpler and more convenient. However, its maturation requires greater effort and appears likely to escalate as a popular process in several traditional and new detection techniques. Undoubtedly, the development of aptamers endows this technique with more potential in future bioassays, due to the unique properties of aptamers.

As a matter of fact, most developed aptasensors have been designed in an ALF mode. Generally, the aptamers need to be assigned the responsibility to both recognize and produce signals. This means the detectable signals are produced mainly depending on the labeled probes on aptamers or other changes that aptamers undergo (e.g., conformational change) in the presence of targets. First, like some label-free immunoassays, this achieves detection while avoiding target destruction after labeling and holds the affinity at a higher degree. Second,

TABLE 12.1 Advantages of Label-Free Detection

Studying molecular interactions without the relatively complex step of modifying molecules and thus simplifying the experimental process
Experiencing the influence of label on physicochemical or binding properties and thus retaining higher levels of activity and affinity
Avoiding problems caused by secondary detection or auxiliary reagents and thus increasing sensitivity and specificity
Providing the potential for assays to be cheaper (no labeling or reagent costs)
Shortening assay development times, thus reducing the time required to complete detection

TABLE 12.2 Submodes in the Analyte Label-Free Mode

	SOALF Mode		SFALF Mode	
	POSOALF Mode	PFSOALF Mode	POSFALF Mode	PFSFALF Mode
Substrate to immobilize[a]	On	On	Off	Off
Signal probe labeling[b]	On	Off	On	Off
Smart degree[c]	*	**	**	***

[a]The aptamers need to be fixed onto certain types of substrates for recognizing, separating, and enriching targets or providing a collection platform for signals.
[b]Aptamers will be labeled with certain probes to produce detectable signals.
[c]The more asterisks there are, the smarter the mode is.

complicated steps of chemical modification on targets are not necessary, leading to relatively easier operations.

As a step further, ALF could be divided into the following modes, which are listed in Table 12.2.

1. *Substrate-on ALF (SOALF).* Concretely, despite the term *analyte label-free*, the aptamers need to be fixed onto certain types of substrates to recognize, separate, and enrich targets or to provide a collection platform for the signals. Then, depending on the methods producing the signals, SOALF will be separated into two modes:
   a. *Probe-on SOALF (POSOALF).* For this type of sensor, aptamers will not only be fixed but will also be labeled with probes to produce detectable signals.
   b. *Probe-off SOALF (PFSOALF).* For this mode, signal probes will also be cut off. The signals are produced primarily based on changes in the sensing surface in the presence of targets (e.g., conformational changes, charge changes).
2. *Substrate-off ALF (SFALF).* As a further simplified mode, substrates for immobility are not excluded. In this type of sensor, sensing systems are more smartly designed, and detectable signals are delivered only through disturbances resulting from the aptamer–target binding process. Obviously, it is a more convenient mode to use as an apsensor. Again, two discrete modes are included.
   a. *Probe-on SFALF (POSFALF).* Similar to POSOALF, probes need to be labeled onto the aptamers to produce signals.
   b. *Probe-off SFALF (PFSFALF).* This is the simplest mode of all: no fixation, no separation, no label, and no probe.

Now, in conjunction with Table 12.2, we introduce these easy label-free modes in detail to help us to understand how useful aptamers are in making detection

simple and convenient. In terms of their degree of smartness, most attention will be focused on POSFALF and PFSFALF, especially PFSFALF.

It should be noted that there is another type of in vitro functional oligonucleotides selected: deoxyribozymes/ribozymes (DNAzymes/RNAzymes) (Breaker and Joyce, 1994, 1995; Cuenoud and Szostak, 1995; Li and Sen, 1996; Breaker, 1997; Sen and Geyer, 1998). Different from the aptamers, they are catalytic nucleic acids capable of catalyzing a broad range of reactions, including cleaving nucleic acid substrate (Breaker and Joyce, 1994, 1995), ligation (Cuenoud and Szostak, 1995), phosphorylation (Li and Breaker, 1999), and porphyrin metallation (Li and Sen, 1996). Because they have also been in common use for bioassays in recent years, sensors adopting these DNAzymes/RNAzymes are included. In the following section we use the word *aptasensors* to represent sensors that use either aptamers or DNAzymes/RNAzymes.

12.2 ELECTROCHEMICAL APTASENSORS

Electrochemical aptasensors have been developed widely for some period of time, especially in the most recent few years (Willner and Zayats, 2007). Due to the properties of the technique itself, most electrochemical aptasensors depend on electrodes for electron transfer. Thus, the SOALF mode could make sense. Here we introduce mainly easy routes in this mode rather than in the SFALF mode.

12.2.1 POSOALF Mode

Most electrochemical aptasensors reported belong to this mode, for it requires redox probes to produce electrochemical signals on the electrode surface. Wang's group has employed an indirect amplified electrochemical route using aptamer–protein complexes binding with inorganic nanocrystals as labels and has accomplished the multianalysis of various proteins by their specific aptamers (Hansen et al., 2006). A gold electrode is functionalized with aptamers specific to α-thrombin and lysozyme. The α-thrombin and lysozyme are modified with CdS and PbS quantum dots (QDs), respectively, and both are bound to the corresponding aptamers associated with the surface. The QD-functionalized proteins acted as tracer labels for analysis of the proteins. In the presence of nonfunctionalized α-thrombin or lysozyme, displacement of the corresponding labeled proteins followed by dissolution of the remaining QDs captured on the electrode surface, and then detection by electrochemical stripping of the ions released, enables quantitative detection of the two proteins. As described, QDs here are not taken as stable and strong fluorescence probes but endowed with a novel electrochemical function. Dong's group uses a similar sensing process but for the first time uses the QDs as an amplified electrochemiluminesence (ECL) co-reactant in aptasensors (as shown in Figure 12.1A) (Guo et al., 2008). Using these semiconductor nanocrystals, both lysozyme and α-thrombin are sensitively detected.

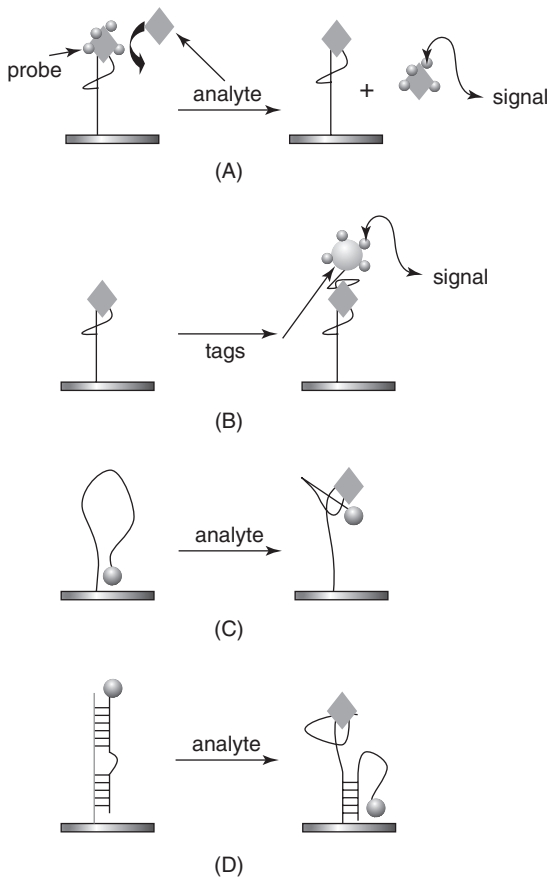

Figure 12.1 Principles of different types of POSOALF. (A) Probe-modified targets will be replaced by free targets and then collected in an electrochemical cell to produce a detectable signal (Guo et al., 2008). (B) Sandwich-type sensors in which aptamer-modified tags are generally used as signal elements (Ikebukuro et al., 2005; Mir et al., 2006; Polsky et al., 2006; Centi et al., 2007; Zheng et al., 2007; Zhou et al., 2007). (C) In the presence of analytes, conformational changes in aptamers will change the distance between the probe and the electrode. Then detectable electrochemical signals can be produced (Xiao et al., 2005). (D) TREAS mode. After adding analytes, the duplex will be destroyed and the modified probe will approach the electrode. Then an increased electrochemical signal can be detected (Xiao et al., 2005; Zuo et al., 2007).

Sandwich-type sensing platforms are also used widely in electrochemical aptasensors (Willner and Zayats, 2007), especially for common model molecules such as α-thrombin (Mir et al., 2006; Polsky et al., 2006; Centi et al., 2007) and platelet-derived growth factor (PDGF) (Zhou et al., 2007), which possess two active aptamer-binding sites. This type of sensor usually

contains an aptamer-modified electrode as a detection substrate and another aptamer-modified electrochemical active material as a signal-producing probe (Figure 12.1B).

Until now, a variety of probes, such as horseradish peroxidase (HRP) (Mir et al., 2006) pyroquininoline quinone glucose dehydrogenase (PQQ-GDH) (Ikebukuro et al., 2005), and nanoparticles (Polsky et al., 2006; Zheng et al., 2007), have been used in sandwich-type sensors. For example, a sensing platform is constructed by immobilizing one α-thrombin aptamer (15-mer) onto a gold electrode for capturing the target and the other aptamer (29-mer) modified with PQQ-GDH for detection (Zhou et al., 2007). In the presence of α-thrombin, the PQQ-GDH-modified aptamer will interact with the protein and an electrochemical signal is produced after the addition of glucose. In this way, as little as 10 nM α-thrombin can be detected selectively and a linear response is obtained between 40 and 100 nM.

Obviously, the sensors above are fabricated relatively complicatedly, because most sensors need multiple steps of modification, label, or separation. These steps might display their advantages, such as high selectivity and sensitivity, but still not avoid the defaults that label usually faces. As a matter of fact, most electrochemical aptasensors have been designed for speed and simplicity, which exemplify the inherent advantages of electrochemistry. Hence, most electrochemical aptasensors reported are generally simpler.

An original label-free electrochemical aptasensor is derived from the electronic aptamer-based (E-AB) sensors of Heeger and Plaxco (Xiao et al., 2005a). As shown in Figure 12.1C, this sensor is designed for α-thrombin, and the signal generation depends only on obvious, target binding–induced aptamer conformational change on a redox-aptamer-modified electrode. The aptasensor developed is sensitive and reusable. More important, it does not require modification of the analyses (Xiao et al., 2005a). Although this type of E-AB does not avoid the probe-label process completely, it does release analytes from modification or relatively complicated experiment steps. In successive works, a series of electrochemical aptasensors have been developed based on this principle, with the targets extended to other proteins (Lai et al., 2007), small molecules (Baker et al., 2006), and even metal ions (Xiao et al., 2007). The probe was extended to ferrocene as well by O'Sullivan's group (Radi et al., 2006). The signal-produced mode is also changed to improve the sensing efficiency according to the different targets.

As a further step, a target-responsive electrochemical aptamer switch (TREAS) was explored. TREAS is, in fact, a duplex-to-complex mode usually containing a duplex or partial duplex hybridized between an aptamer-containing strand and its complementary or partly complementary strand (Xiao et al., 2005b; Zuo et al., 2007). Under given conditions, these duplexes could be split in the presence of targets and induce a detectable signal change. The advantages of TREAS rest on the fact that they can be designed smartly to improve the sensors' performance and are widely adopted in various electrochemical methods. For example, the initial E-AB is improved from a signal-off to a signal-on sensor; thus, a one-order

increase in sensitivity can been obtained (Figure 12.1D) (Xiao et al., 2005). Fan and co-workers have realized small-molecule ATP detection using a TREAS aptasensor (Zuo et al., 2007).

12.2.2 PFSOALF Mode

In this mode redox probes to produce detectable signals are no longer covalently labeled onto the aptamers in this mode. Probes dissolved in electrolyte solution can interact with aptamers immobilized on an electrode via (1) electrostatic repellence (for negatively charged probes), (2) electrostatic adsorption (for positively charged probes), and (3) intercalation (for DNA intercalators).

12.2.3 Electrochemical Impedimetric Aptasensors

Electrochemical impedance spectroscopy (EIS) is a rapidly developing electrochemical technique that has been incorporated in the design of biosensing systems to utilize its advantages, which include sensitivity, low cost, and convenience, as well as being label-free (Katz and Willner, 2003; K'Owino and Sadik, 2005; Pejcic and De Marco, 2006; Daniels and Pourmand, 2007). As noted by Radi et al. (2005), "EIS is an electrochemical technique for the investigation of bulk and interfacial electrochemical properties of any type of solid or liquid material connected to or part of an appropriate electrochemical transducer. Any intrinsic property of a material or a specific process that could affect the interfacial properties of an electrochemical system can potentially be studied by EIS." That is why EIS is popular in label-free sensor fabrication. This technique is well suited to monitoring the various stages necessary for characterizing and detecting the recognition event when an immobilized molecule interacts with its ligand (the analyte).

Aptamer-based impedimetric bioassays are developing rapidly. In 2005, two papers in this field published in the same issue of *Analytical Chemistry* (Radi et al., 2005; Xu et al., 2005). They brought EIS into an aptasensing process and gave simple PFSOALF examples of electrochemical detection. These aptamer-based impedimetric bioassays are designed to focus on protein detection in a general electrode–aptamer–target mode (the principle is illustrated in Figure 12.2A).

In Radi's work, a gold electrode is functionalized with thiolated α-thrombin aptamer (TBA) and blocked with neutral 2-mercapoethanol (MCE) to act as a sensing interface for α-thrombin (Radi et al., 2005). This negatively charged interface repels the anionic redox couple of $[Fe(CN)_6]^{3-/4-}$ and therefore repels the electron-transfer process. The electron-transfer resistance (Ret) produced is greater than that of bare electrodes. For α-thrombin the pH is nearly neutral, about 7.5, with a molecular mass of about 35,000 Da. When it binds its aptamer on the electrode here, the Ret increases due to an integrated function of both its bulk size and the resistive hydrophobic layer insulating the conductive support. The protein is detected simply by this further increase in electron transfer impedance (Ret), with a sensitive detection limit of about 5 nM. Similarly, in Xu's work,

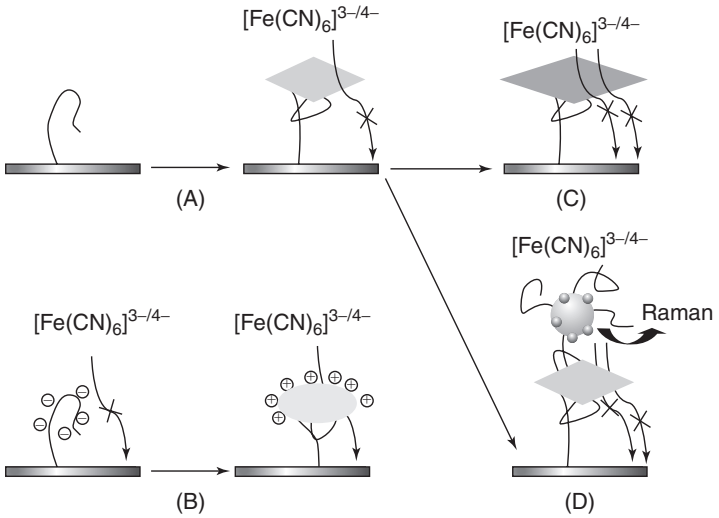

Figure 12.2 Principles of different types of impedimetric aptsensors: (A, B) the simplest modes for protein detection; (C) denaturalization by guanidine hydrochloride to amplify the signal; (2006); (D) scheme of a sandwich-type sensor. Aptamer/R6G-modified AuNPs are used as amplifying elements. [(A) from Radi et al. (2005); (B) from Rodriguez et al. (2005); (C) from Xu et al. (2006); (D) from Wang et al. (2007); Li and Dong (2008).]

using the same principle, immunoglobulin E (IgE) is taken as the target (Xu et al., 2005). The difference lies in the fact that the sensing surface is constructed on an array configuration of electrodes, which provides the ability to examine the effect of different base mutations in the aptamer sequence on the affinity for IgE–aptamer binding.

At almost the same time, another impedimetric aptasensor was developed using lysozyme as an analyte (Figure 12.2B) (Rodriguez et al., 2005). Despite the same electrode–aptamer–target method being used, the result is different. A negative sensing surface to repel a $[Fe(CN)_6]^{3-/4-}$ probe is generated through a biotinylated aptamer linked to a streptavidin-functionalized indium tin oxide electrode. Then the association of protein leads to a Ret decrease rather than an increase. That is attributed to the positive charge exhibited by lysozyme at about pH 7. The +8 net charges are high enough to provide an excess positive charge, which facilitates access of the probes and the resulting redox reaction. The impedimetric aptasensors described above are the simplest to use for protein detection. Recently, the design has been improved by a series of amplified methods to realize higher sensitivity (Xu et al., 2006; Li and Dong, 2008). Taking α-thrombin as a model molecule, Fang's group added guanidine hydrochloride disposal after target binding (Figure 12.2C) (Xu et al., 2006). It was found that protein denatured by guanidine hydrochloride can remain on the electrode and

lead to a further enhanced Ret signal. Due to this smart design, about a 10-fold higher sensitivity than that obtained without amplification is achieved.

Another example comes from Dong's group (Li and Dong, 2008). For α-thrombin detection, this work is based on the fact that one α-thrombin molecule has two active sites for its 15-mer aptamer. As shown in Figure 12.2D, gold nanoparticles (AuNPs) are first functionalized by aptamers for recognizing protein and rhodamine 6G (R6G) molecules for blocking the surface. During the sensing process, thiolated α-thrombin-binding aptamer is first immobilized on the gold electrode to capture the target, and once in the presence of the α-thrombin, the bifunctionalized AuNPs could bind further to α-thrombin, forming a sandwich-type sensing system on the electrode. For negatively charged bifunctionalized AuNPs, the Ret signal is obviously amplified (Figure 12.3) (Li and Dong, 2008).

Through such an amplified method, a detection limit of 0.02 nM is realized. In fact, a disadvantage for impedimetric sensing is that the detection is easily interfered with by the nonadsorption of other materials on the electrode. So here, R6G molecules modified on AuNPs can provide a very selective surface-enhanced Raman scattering (SERS) method to realize qualitative recognition using gold nanoparticle–induced "hotspots" (as shown in Figure 12.4) (Wang et al., 2007). As shown, although it belongs to the POSOALF mode, the foregoing advantages still provide the potential to carry out mixed-technique analysis more easily.

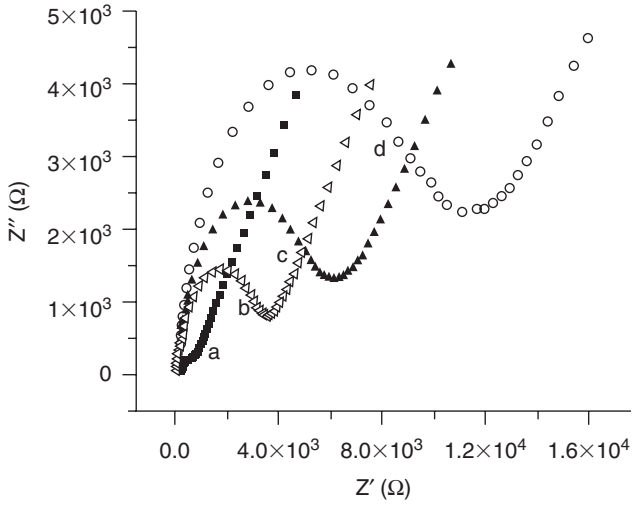

Figure 12.3 Niquist plots of (a) a bare Au electrode, (b) an Au/TBA/MCE system, (c) an Au/TBA/MCE/α-thrombin system, and (d) an Au/TBA/MCE/α-thrombin/AuNPs system. The concentration of α-thrombin was 45 nM. TBA, α-thrombin-binding aptamer; MCE, blockers modified after the TBA, 2-mecaptoethanol). [From Li and Dong (2008), with permission. Copyright © 2007 Elsevier.]

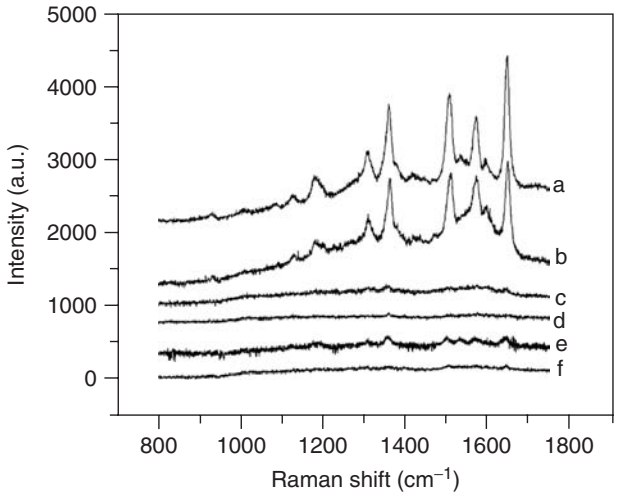

Figure 12.4 SERS spectra of Raman reporters with various proteins. (a) 100 nM α-thrombin. (b) 200 nM β-thrombin, 200 nM γ-thrombin, and 100 nM α-thrombin. (c) 200 nM β-thrombin and 200 nM γ-thrombin. (d) 400 nM β-thrombin. (e) 1 μM BSA and (f) without protein. [From Wang et al. (2007), with permission. Copyright © 2007 Royal Society of Chemistry.]

Undoubtedly, bulk proteins are very suitable targets for impedimetric aptasensors. However, the conditions are not the same for small molecules because most small molecules have a low molecular mass or low net charge that is not strong enough to affect conditions on the electrode surface.

Recently, strategies to overcome the shortcomings noted above have been developed to make EIS detection available for small molecules. Usually, the TREAS mode is needed. In one of the strategies, TREAS is designed with a part-duplex DNA consisting of an amine-functionalized aptamer–containing strand and its partially complementary strand (Figure 12.5A) (Zayats et al., 2006). Linked by dithiobis (succinimidylpopionate), the part duplex is immobilized onto a gold electrode through the amine group on the apatmer-containing strand. In the presence of analytes, the part duplex is separated due to aptamer–target interaction, leading to loss from the electrode of the partially complementary strand. The disassociation results in removal of the negative charge from the electrode surface and thus decreased interfacial electron-transfer resistance in the anion redox couple $[Fe(CN)_6]^{3-/4-}$. Taking AMP as a model, fast and sensitive detection is realized, with a response time of less than 5 minutes and a detection limit of 2×10^{-6} M. By such a TREAS mode, EIS signals are not transduced through the analytes themselves but through the changes in TREAS after binding with the analytes. It is clear that smart concepts will ultimately extend the EIS technique to wider applications.

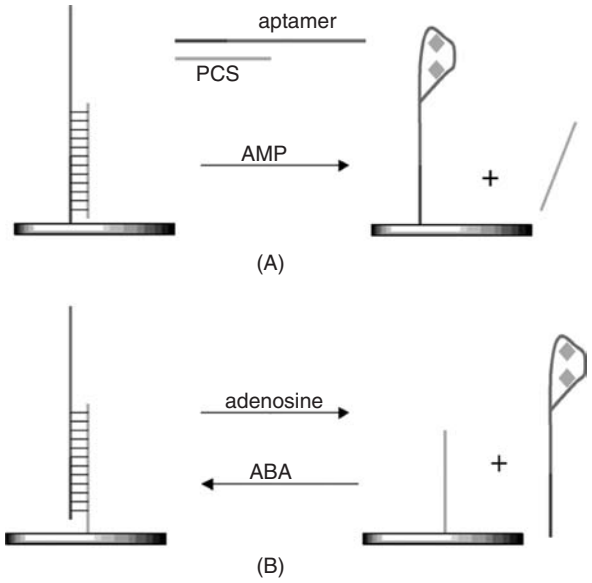

Figure 12.5 Principles of the two types of impedimetric aptasensors in TREAS mode. (A) An aptamer-containing strand is immobilized onto the electrode (B) A partlially complementary strand (PCS) is immobilized onto the electrode ABA represents the adenosine-binding aptamer. [(A) From (Zayats et al. 2006); (B) from (Li et al. 2007b).]

Dong's group developed a different but also interesting strategy (Figure 12.5B) (Li et al., 2007b). As an opposite route to the design described above (Figure 12.5A), it is the partially complementary strand that is immobilized on a gold electrode (through thiol modified on the strand), not the apatmer-containing strand (for adenosine). Correspondingly, when analytes are present, the aptamer-containing strand would fall off the electrode, which leads to decreased interfacial electron-transfer resistance in the anion redox couple $[Fe(CN)_6]^{3-/4-}$ (Figure 12.6A). The detection limit for adenosine in this work is as low as 5×10^{-7} M. Such a higher sensitivity compared to that of the sensor described above (Zayats et al., 2006) may be attributed to loss of the relatively longer aptamer-containing strand. This is just one of the advantages of this strategy (Li et al., 2007b). Meanwhile, the sensing interface is also endowed with regenerative ability by direct rehybridizing with the aptamer-containing strand. Through testing, the sensing interface can be recovered to more than 90% after one detection (Figure 12.6B). It is notable that the method proposed does not rely on molecule size or the aptamer's conformational change, so it may possess a potential for the wider application of more targets, even proteins.

Recently, such a strategy has been extended to protein detection (Du et al., 2008). In this technique a unimolecular DNA strand containing two types of aptamers for different analytes is designed. As an example, two aptamers are

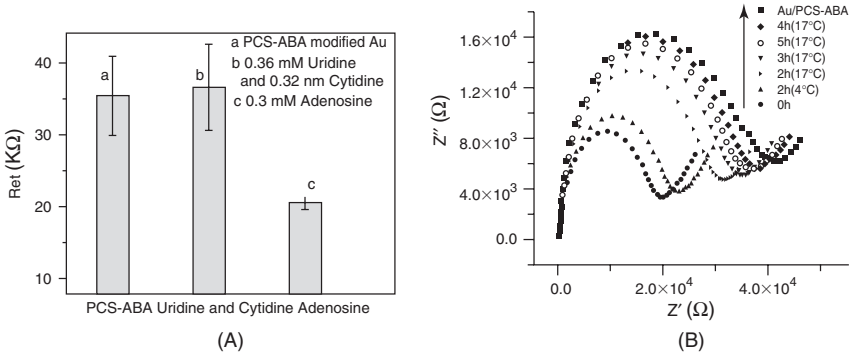

Figure 12.6 Comparison of Ret between 0.3 mM adenosine (c) and a mixed solution of control molecules (0.36 mM uridine and 0.32 mM cytidine) (b). In the presence of adenosine, obvious Ret decrease is observed; in the presence control molecules, no decrease is observed. The error bars represent the standard deviation of two measurements. [From Li et al. (2007), with permission. Copyright © 2007 Royal Society of Chemistry.]

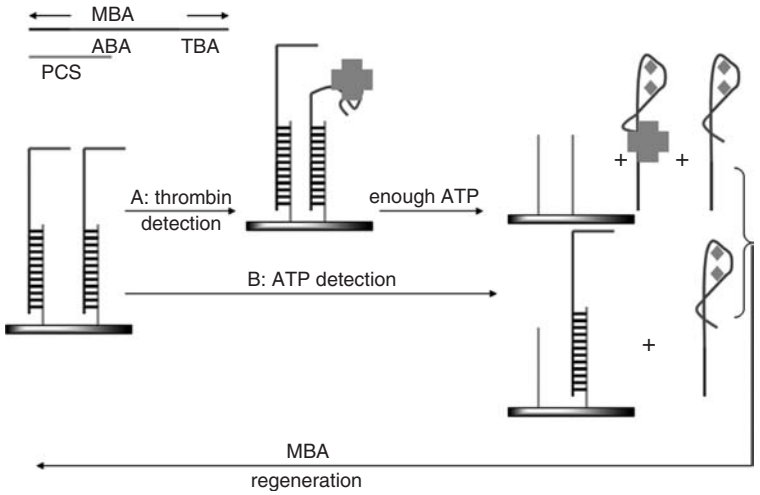

Figure 12.7 Principle of the multifunctional aptasensor. ABA, ATP-binding aptamer; TBA, α-thrombin-binding aptamer; MBA, strand containing the aptamers; PCS, strand partly complementary to MBA. [From Du et al. (2008).]

chosen for ATP and α-thrombin and combined in a unimolecular DNA strand called a mixed binding aptamer (MBA) (Figure 12.7). In this MBA, the aptamer for ATP is partially hybridized with the partially complementary strand, while the aptamer for α-thrombin is free beside the part duplex. So in the presence of the protein α-thrombin, the interfacial electron-transfer resistance (in the anion

redox couple $[Fe(CN)_6]^{3-/4-}$) is increased as explained earlier, which could be used to recognize the analyte. Furthermore, a sensitive detection limit of 1×10^{-11} M is achieved. However, the specialty of this sensor rests with its regeneration method and potential for multianalysis. After the protein has been detected, the sensing surface is treated with a large amount of ATP. Following the principle introduced above, enough ATP could draw all the MBA (containing that bound with α-thrombin) away from the sensing surface and prepare it for regeneration. This process may also be used to detect ATP when treating protein-covered electrode with the required concentration of ATP. Correspondingly, after treatment with ATP, the electrode could be recovered again using MBA (Figure 12.7). However, such a fabricated sensing system could not be used for samples with multiple analytes in one solution. So improvements are still needed.

12.2.4 Electrochemical Aptasensors with Nonlabeled Redox Probes

An electrochemical sensor with a nonlabeled redox probe shakes off probes labeled on the apatmers but still requires that the probes indicate changes on the sensing interface. One commonly used probe, methylene blue (MB), belongs to the phenothiazine family and is an aromatic cationic dye showing optically and electrochemically active properties (Tuite and Norden, 1994). Usually, MB can bind with dsDNA or tRNA via intercalation, electrostatic absorption, or G-base binding, which has been used widely in DNA sensors and recently, in aptasensors (Tuite and Norden, 1994; Bang et al., 2005).

Kim's group uses MB as a probe in a molecule beacon such as the detection of α-thrombin (Figure 12.8A) (Bang et al., 2005). At first, an amino-functionalized ssDNA containing 15-mer α-thrombin aptamer is synthesized in a hairpinlike conformation with five base pairs on the arm. Then the ssDNA is linked to a carboxyl-modified gold electrode, followed by blocking the electrode surface with BSA. Such a modified electrode is treated further with an MB solution to produce an MB intercalated DNA-functionalized sensing surface. Through differential pulse voltammetry (DPV), a reduction peak of MB incorporated into the arms of the molecule beacon is observed at −0.2 V. In the presence of α-thrombin, more of the hairpinlike aptamer beacons are changed into a G-quadruplex conformation state due to aptamer/α-thrombin interaction. The hairpin structure is thus opened and the MB is released, which leads to a decreased MB current at −0.2 V. Using this sensor, a linear range between 0 and 50.8 nM ($r = 0.999$) is reached, with an estimated detection limit of 11 nM. The results demonstrate that the electrochemical method using an aptamer probe is indeed convenient and allows quantitative detection of target protein.

Using another route, Dong's group realized MB-based small-molecule detection using TREAS. Like the EIS sensor described earlier (Figure 12.5B), a part duplex consists of a partially complementary strand immobilized onto a gold electrode with an aptamer-containing strand for recognition (Figure 12.8B). Taking adenosine, for example, before treatment with the analyte, MB molecules will

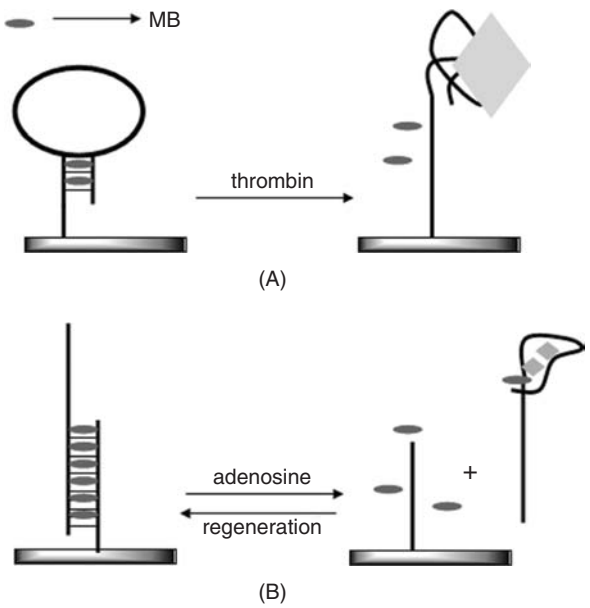

Figure 12.8 Two types of sensors with MB as a probe: (A) aptamer-beacon approach; (B) TREAS approach. [(A) From Bang et al. (2005); (B) from Li et al. (2007b).]

intercalate into the part duplex and produce an obvious current at −0.2 V. Once in the presence of adenosine, the aptamer–analyte interaction will destroy the duplex and reduce the amount of MB intercalating the DNA, which eventually results in decreased current (Figure 12.9). The sensor is sensitive, selective, and could be regenerated for later use.

Another commonly used redox probe, $[Ru(NH_2)]_6^{3+}$, could interact with DNA molecules through electrostatic adsorption and thus be used as an indicator for the amount or density of DNA on an electrode (Yu et al., 2003; Su et al., 2004). Also, protein is detected using $[Ru(NH_2)]_6^{3+}$ as a probe (Figure 12.10A) (Cheng et al., 2007). Antilysozyme DNA aptamers are immobilized on gold surfaces by means of self-assembly, for which the surface density of aptamers is determined by cyclic voltammetry (CV) for redox behavior of cations $[Ru(NH_2)]_6^{3+}$ bound to the surface via electrostatic interaction with the DNA phosphate backbone. Upon incubation of the electrode with a lysozyme solution, the CV response of surface-bound $[Ru(NH_2)]_6^{3+}$ decreases substantially. This results from the fact that the binding of lysozyme to an aptamer-modified gold electrode (with its inherent ability to bind in a 1 : 1 ratio) should reduce the negative surface charges (contributed by the DNA backbone) since lysozyme has a net charge of +8. Then the relative decrease in the integrated charge of the reduction peak can be tabulated as a quantitative measure of protein concentration, with a detection limit of 0.5 $\mu g\ mL^{-1}$.

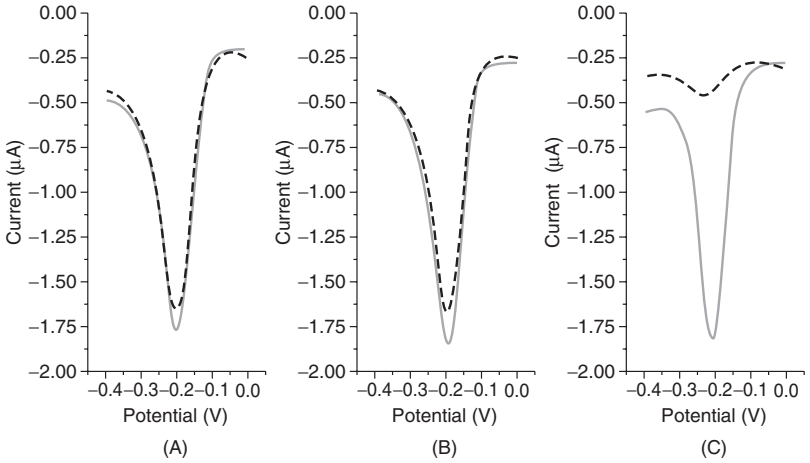

Figure 12.9 Differential pulse voltammetries of the sensing system before (solid line) and after (dashed-dotted line) being reacted with three nucleosides at 1 mM in 25 mM TRIS–HCl solution containing 20 mM NaCl: (A) uridine; (B) cytidine; (C) adenosine. [From Wang et al. (2008), with permission.]

TREAS is also used for small-molecule detection (Figure 12.10B) (Shen et al., 2007). In this case, the detectable signal is produced from discriminate electrostatic interaction between $[Ru(NH_2)]_6^{3+}$ and the part-duplex or aptamer-containing strand. A chronocoulometric technique is used to indicate the result. So when an analyte (AMP) exists, the signal is reduced by the amount by which $[Ru(NH_2)]_6^{3+}$ confined on the electrode surface decreased. Recently, a ferrocene-functionalized cationic polyelectrolyte poly(3-alkoxy-4-methylthiophene) was used as a novel redox probe (Le Floch et al., (2005)). Thiolated α-thrombin-binding aptamer is linked to an Au electrode. The electrostatic interaction of the polyelectrolyte with the aptamer yields a voltammetric response in the ferrocene. After binding with α-thrombin, the cationic polymer is blocked for proteins that are nearly neutral under binding conditions, so the electrochemical response is reduced. Although it is convenient to operate, the detection limit of this sensor is not satisfactory, being higher than 10^{-6} M.

12.3 FLUORESCENT MOLECULAR SWITCHES

Fluorescence spectroscopy is a traditional technique for fabricating biosensing systems and has been used widely in aptasensors for small molecules (Stojanovic et al., 2000a, 2001; Nutiu and Li, 2003), proteins (Hamaguchi et al., 2001; Yang et al., 2005; Li et al., 2007a), metal ions (Li and Lu, 2000; Lu et al., 2003; Nagatoishi et al., 2005; Shen et al., 2007), and even cells (Herr et al., 2006; Shangguan et al., 2006).

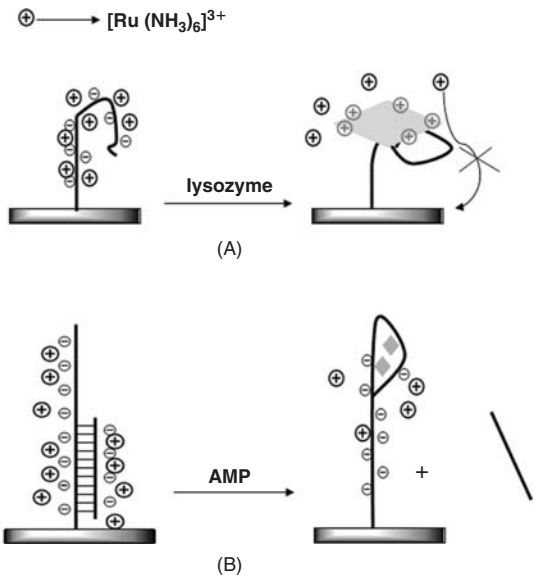

Figure 12.10 Two types of sensors with $[Ru(NH_2)]_6^{3+}$ as a probe: (A) Aptamer beacon approach; (B) TREAS approach. [(A) From Cheng et al., (2007); (B) from Shen et al. (2007).]

Until now, several strategies have been reported for converting an existing aptamer into a fluorescent probe, among which the methods used most frequently are the molecular beacon (aptamer beacon) approach (Figure 12.11A) and the duplex-to-complex switching approach (Figure 12.11B) (Navani and Li, 2006). Both approaches profit from the inherent properties of aptamers as oligonucleotide molecules. Thus, most aptasensors with fluorescence detection can be prepared smartly, which means that in SFALF modes, general steps such as separation and immobilization can easily be left out.

12.3.1 POSFALF Mode

Although in the SFALF mode, a majority of fluorescent aptasensors are still "probe-on" (POSFALF), which mode is the most popular for use in fluorescent assays. The aptamer beacon approach can often be divided into two commonly adopted aspects. One, like a molecular beacon for DNA sensing, places an aptamer sequence in a molecular beaconlike hairpin structure end-labeled with a fluorophore (F) and a quencher (Q) (Figure 12.11A) (Hamaguchi et al., 2001; Yamamoto and Kumar, 2000; Lu et al., 2003; Nagatoishi et al., 2005; Li et al., 2007; Shen et al., 2007) the binding of the target disrupts the hairpin stem and separates F from Q, leading to a detectable increased fluorescence. The other aspect often labels the aptamer with F1 and Q/F2 at each end, respectively

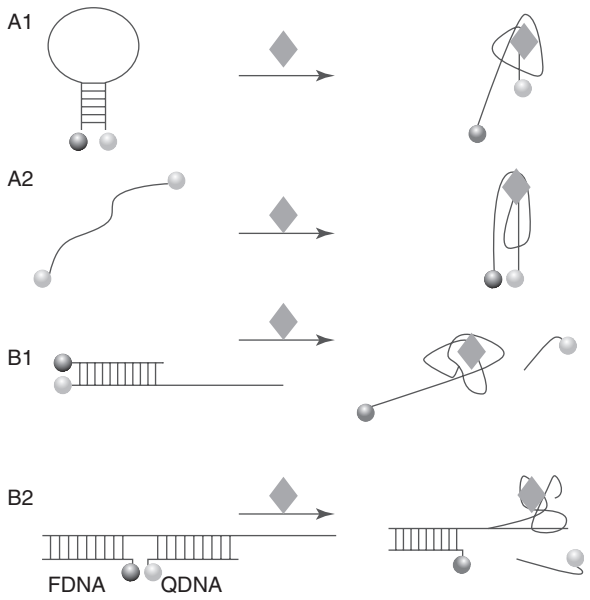

Figure 12.11 Two approaches to designing POSFALF fluorescent aptasensors. The aptamer beacon approach contains two modes of signal-on (A1) (Yamamoto and Kumar, 2000; Hamaguchi et al., 2001) and signal-off/FRET (A2) (Li et al., 2002; Ueyama et al., 2002). The duplex-to-complex switching approach contains two signal-on modes: B1 (Rupcich et al., 2005; Elowe et al., 2006) and B2 (Nutiu and Li, 2003).

(Figure 12.11A) (Li et al., 2002; Ueyama et al., 2002). It requires only conformational change of aptamers once they bind to targets to get a quenched or fluorescence resonance energy transfer (FRET) signal.

The duplex-to-complex switching approach (Figure 12.11B) is usually divided into two modes. One often places an F-labeled (Q-labeled) aptamer in a duplex structure with a (partial) complementary sequence labeled with a Q (F) (Figure 12.11B1) (Rupcich et al., 2005; Elowe et al., 2006). Addition of the target forces the departure of the partial complementary strand from the aptamer, accompanied by an increase in fluorescence. In the other mode, one often needs to design a duplex consisting of one aptamer-containing sequence and two partially complementary strands that are labeled with F (FDNA) and Q (QDNA), respectively, on the ends near each other (Figure 12.11B2) (Nutiu and Li, 2003). When target in present, the QDNA will be completed, due to the formation of an aptamer–target complex, and thus an increased fluorescence is observed. A representative aptasensor fabricated in this way was developed by Li's group, in which both small molecules of ATP and proteins of α-thrombin were detected successfully (Figure 12.11B2) (Nutiu and Li, 2003). Obviously, in comparison to the aptamer beacon approach, the duplex-to-complex switching

approach is a little more complex but appears to be more applicable, for in this way the dependence on the design of a hairpin structure or conformational change of aptamers is reduced, which exposes it to wider targets. However, the sequences of duplexes in this approach should be more carefully optimized to guarantee a satisfactory sensing efficiency and high signal/noise ratio. Now, through continuous improvement, the duplex-to-complex switching approach has been used successfully in enzyme-related assays (Rupcich et al., 2006) and DNAzyme-related assays (Stojanovic et al., 2001b).

Recently, with the development of synthesis techniques, new fluorescent probes are employed continually as an alternative to commonly used F or Q to decrease the background or improve the practicality (Nagatoishi et al., 2005; Yang et al., 2005). For example, Tan's group has labeled PDGF-binding DNA aptamer with one pyrene molecule on each end to produce a time-resolved aptamer beacon (Yang et al., 2005). Upon PDGF binding, the aptamer switches its fluorescence emission from 400 nm (pyrene monomer, with a fluorescence lifetime of about 5 ns) to 485 nm (pyrene excimer, with a fluorescence lifetime of about 40 ns). Such wavelength shifting, especially the time-resolved capabilities, would benefit practical samples by decreasing the background signals coming from the native fluorescence of the biological environment (Yang et al., 2005).

Novel nanoparticles such as QDs are also used (Dwarakanath et al., 2004; Levy et al., 2005; Choi et al., 2006; Ikanovic et al., 2007; Liu et al., 2007). Ellington's group fabricated a duplex-to-complex switching aptasensor by labeling α-thrombin-binding aptamer with QDs and the complementary strand with Q, which could quench the fluorescence of QDs before sensing (Levy et al., 2005). The addition of α-thrombin will withdraw the QDs from Q, leading to a recovered fluorescence.

12.3.2 PFSFALF Mode

Although labeled probes might produce a higher signal-noise ratio and relatively wider applications in fluorescent aptasensors, smarter routes with no label steps are also promising. Their advantages include convenience, low cost, ease of preparation, and a sensitivity similar to that of labeled methods.

"We report the first examples of modular aptametric sensors which transduce recognition events into fluorescence changes through allosteric regulation of noncovalent interactions with a fluorophore" (Stojanovic and Kolpashchikov, 2004). These sensors are designed to access the PFSFALF mode in fluorescent aptasensing, which makes them readily applicable for intracellular applications. As shown in Figure 12.12, the main sensing systems contain three parts: (1) aptamer of analyte (ATP, etc.) as a recognition domain, which binds the analyte; (2) RNA aptamer of malachite green (MG, a dye) as a reporting domain, which increases the quantum yield of this dye up to 2000-fold upon binding and is thus used to signal the binding event of an analyte through binding to a fluorophore; and (3) a communication module, which is designed as a conduit

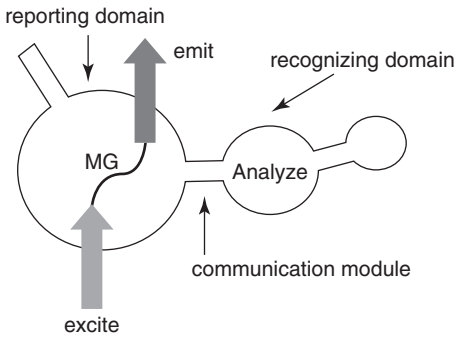

Figure 12.12 Parts of the sensing system: a recognizing domain to bind analyte, a reporting domain to bind MG molecule, and a communication module to combine the recognizing domain and the reporting domain. Induced by the interaction between analyte and its aptamer, the fluorescence intensity ($\lambda_{ex} = 610\,nm$) of MG will be enhanced. [From Stojanovic and Kolpashchikov (2004).]

between the recognition and signal domains. Once in the presence of analyte, the recognition process (at the recognition domain) will be transduced through a communication module to the reporting domain, leading to increased recognition of MG and concomitant fluorescence enhancement. By detecting increased fluorescence, ATP and flavine mononucleotide (FMN) are detected successfully and theophylline (TH) is distinguished from its closely related molecule, caffeine. Meanwhile, for better sensing, the communication module is carefully optimized for its key role in signal transduction. It is also proven that all the sensing systems are feasible in mimicking intracellular milieu, but only the MG-FMN sensor shows an impressively stronger signal than that of any of the aptameric sensors reported previously. Even so, the smart method shows the potential worth of greater efforts to use this sensor.

In fact, aptamers selected capable of reporting domains are limited. Another series of fluorescent aptasensors take different routes and serve as complementary ways to make the PFSFALF more easily prepared and available for wider applications. This type of sensor is more similar to the POSFALF mode and can also be divided into two parts: the aptamer beacon approach and the duplex-to-complex switching approach.

Using the aptamer beacon approach depends mainly on conformational changes in aptamers once binding to targets. In an example reported by Bai's group, they take $[Ru(phen)_2(dppz)]^{2+}$ (its structure is shown in Figure 12.13A) as a light switch complex and detect such proteins as IgE, α-thrombin, and PDGF-BB (Jiang et al., 2004). $[Ru(phen)_2(dppz)]^{2+}$ has a high binding affinity to duplex nucleic acid ($K_d, \sim 10^6\ M^{-1}$). It has no luminescence (it is excited at 450 nm) in aqueous solution, as the triplet MLCT (metal-to-ligand charge transfer) excited state is effectively quenched by the hydrogen in the ligand. When it binds to dsDNA, the interaction between the ligand and the base pairs

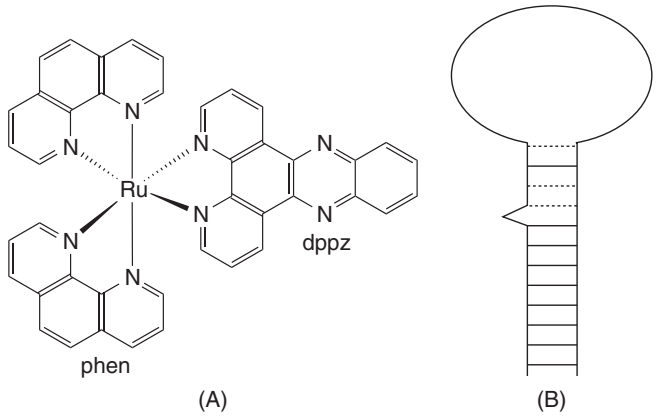

Figure 12.13 (A) Structure of $[Ru(phen)_2(dppz)]^{2+}$; (B) secondary structure of IgE aptamer. Dashed lines represent the non-Waston–Crick base pairs. [From Jiang et al. (2004).]

of duplex nucleic acid protects the phenazine nitrogen from water, leading to an emission at 610 nm up to 10^4-fold higher. Based on this principle, IgE and its 37-mer aptamer are at first chosen as a model system. As reported, IgE aptamer here has a predicted stem–loop structure (Figure 12.13B), including nine Waston–Crick base pairs and three non-Waston–Crick base pairs, which makes it suitable to bind with the dye and thereby leads to a 20-fold emission increase (the dye/DNA ratio is 8 : 1). When the protein is added to the dye–aptamer solution, a significant luminescence intensity decrease is observed within 3 minutes. The reason for the decrease is hypothesized as being that upon protein binding the aptamer conformational change induced, as well as the blocking of $[Ru(phen)_2(dppz)]^{2+}$ intercalation by protein, would result in reduced dye molecules intercalating with the aptamer and thus in a significant protein-dependent fluorescent change. Through the fluorescent change, sensitive and simple detection is realized, with an IgE detection limit of 500 pM in the physiological buffer and almost no disturbance from other proteins.

"The method . . . importantly was applicable to RNA aptamers which have a larger population than DNA aptamers" according to Bai and co-workers (Jiang et al., 2004). Indeed, that may be the most notable advantage for this route and most other nonlabeled sensors for "most of the signalling aptamers developed so far are labeled DNA aptamers, but the fluorescent labelling of RNA aptamers, which have a larger population than DNA aptamers, is difficult because of the instability of RNA molecules" (Jiang et al., 2004). In the same work, detection in human serum is also undertaken. It is found that the detection efficiency in the 1% serum solution remains similar to that in PBS solution, but is much decreased in more concentrated serum solution because of the higher background caused by tailing of broad serum peak around 520 nm. That is just one of the challenges

that most PFSFALF routes should confront and try best to resolve when related to practical detections.

Other systems of α-thrombin/RNA aptamer, PDGF-BB/DNA aptamer, are also proven viable in this work (Jiang et al., 2004) and the strategy is then used for small molecules such as ATP (J. Wang et al., 2005). As a further step, other dyes, such as TOTO, are also employed to improve the method (Zhou et al., 2006). A simple route has been fabricated in a duplex-to-complex switching approach by Dong's group (Li et al., 2007a): "... In principle, if the aptamers did not contain a secondary structure into which dyes intercalated, this method could be applicable." That is one of the aspects that makes a duplex-to-complex switching approach promising.

In this strategy, an α-thrombin/15-mer DNA aptamer system is taken as a model and ethidium bromide (EB) is used as a fluorescing molecular switch. The mechanism of the interaction between the dye and DNA has been delineated clearly. Following the neighbor exclusion principle, where every second site along the helix is unoccupied, EB could readily intercalate between the base pairs of dsDNA. When it is dissociative in aqueous solution EB, $[Ru(phen)_2(dppz)]^{2+}$ shows a low fluorescence intensity attributed to efficient quenching of the excited state by transferring an amino proton to a solvent (water) molecule. Whereas when EB intercalates to dsDNA it is shielded to some extent and shows obvious enhancement in fluorescence intensity (up to nearly 11 times that in its free state), a similar property is not evident when it is mixed with ssDNA or quadruplex DNA, especially with a 15-mer quadruplex, which is simply a more stable structure (than an unfolded conformation) formed when an anti-α-thrombin aptamer binds to α-thrombin. The design is based on this property. As shown in Figure 12.14A, a 15-mer anti-α-thrombin aptamer is hybridized with its complementary strand to form a duplex-to-complex switch. Obviously, in the absence of α-thrombin, an EB molecule intercalates to the DNA duplex with high efficiency, and correspondingly, high fluorescence intensity ($\lambda_{em} = 600$) can be detected. Accompanied by the addition of increasing amounts of α-thrombin, competition between the α-thrombin and the complementary strand for aptamer will lead to partial unwinding of the DNA double-helix structure and a more quadruplex/α-thrombin structure with ssDNA (complementary strands). This, to some extent, releases to the solvent (water) EB being shielded by dsDNA and ultimately makes the fluorescence intensity decrease. As shown in Figure 12.14B, after α-thrombin was added, decreased fluorescent intensity was observed and it took 30 minutes for the α-thrombin/dsDNA/EB system to attain equilibrium. However, if α-thrombin was replaced by a BSA molecule, no fluorescent decrease was observed. Thus, α-thrombin here is detected selectively and sensitively, with a detection limit of 2.28 nM. This method successfully inherits its design concept from labeled duplex-to-complex switching and keeps the majority of advantages that nonlabeled aptamer beacon methods possess. At the same time, it extends targets to systems in which targets have the ability to compete with the complementary strands of their aptamers, or the aptamers do not contain a secondary structure into which EB intercalates.

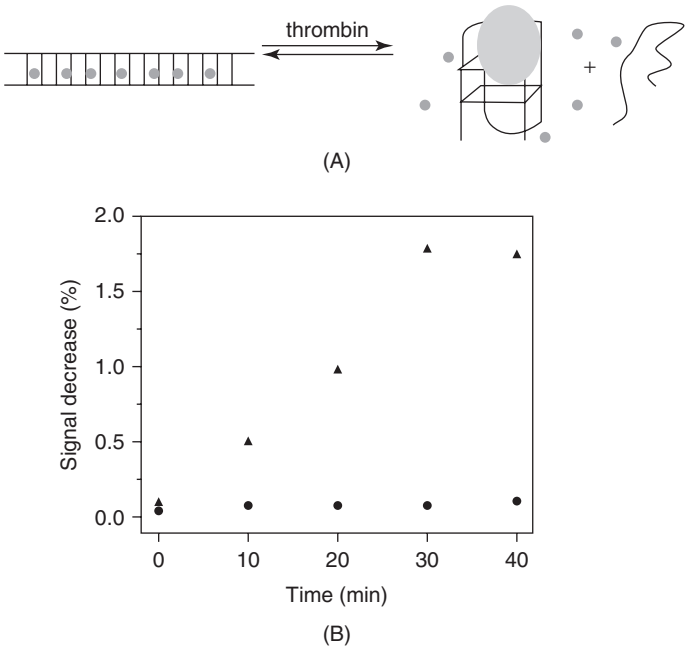

Figure 12.14 (A) Duplex-to-complex switching approach. (B) Time dependence of fluorescence response after a 40-minute incubation. EB/dsDNA/α-thrombin (solid triangles) and EB/dsDNA/BSA (solid dots). [From Li et al. (2007a), with permission. Copyright © 2007 Royal Society of Chemistry.]

The key to the entire response efficiency in this type of sensor is the ability of aptamers to compete with the complementary strand. Although α-thrombin can bind its aptamer with K_d values as low as in the nanometer range, it is still difficult for it to compete with a completely complementary strand to an evident degree, which results in several defaults that limit the detection efficiency: for example, the relatively longer response time (30 minutes) and limited detectable range (to ca. 22.8 nM) (Li et al., 2007a). However, these problems can be resolved by redesigning the duplex-to-complex system and replacing the fluorescing molecular switch EB with other, more effective dyes.

12.4 COLORIMETRY

Due to their simple performance, and especially the potential ability to eliminate the use of analytical instruments, more and more colorimetric sensors are being developed, including DNA sensors (Elghanian et al., 1997), pH sensors (Gazda et al., 2004), metal ion sensors (Ghosh et al., 2006), and now, aptasensors (Huang et al., 2005; Lu and Liu, 2006, 2007; Wang et al., 2006; Zhao et al., 2007).

12.4.1 POSFALF Mode

A large part of colorimetric aptasensors are in this mode. Most of them employ AuNPs as sensing elements. For example, Chang's group has developed a highly specific colorimetric sensing system for PDGF and their reporters (PDGFRs) using AuNPs (Huang et al., 2005). The red dispersed AuNPs are modified using a PDGF-binding aptamer (apt-AuNP) that has two binding sites on one PDGF molecule. Taking PDGF-AA as an example (Figure 12.15A), its addition could draw AuNPs together through one (target) to two (aptamer) binding reactions. That leads to aggregations of AuNPs and a corresponding purple color. At an optimized condition of 8.4 nM apt-AuNPs in 200 mM NaCl, the color changes from red to purple in the presence of 10 nM PDGF-AA, and the linear range calculated by the extinction ratio A_{650}/A_{530} is 25 to 75 nM, with a response time of 60 minutes. Other proteins, such as PDGF-AB, PDGF-BB, and PDGFR-β, are detected sensitively in the same way.

Another series of AuNP-based colorimetric aptasensnors have been fabricated by Lu' s group in a very smart design in which small molecules make use of a duplex-to-complex mode (Lu and Liu, 2006, 2007). In the example shown in Figure 12.15B, AuNPs are reduced to purple aggregates by a duplex consisting of an aptamer-contained chain and two complementary chains in which AuNPs are modified on the 3′ end (3′-$Adap_{Au}$) or the 5′ end (5′-$Adap_{Au}$), respectively

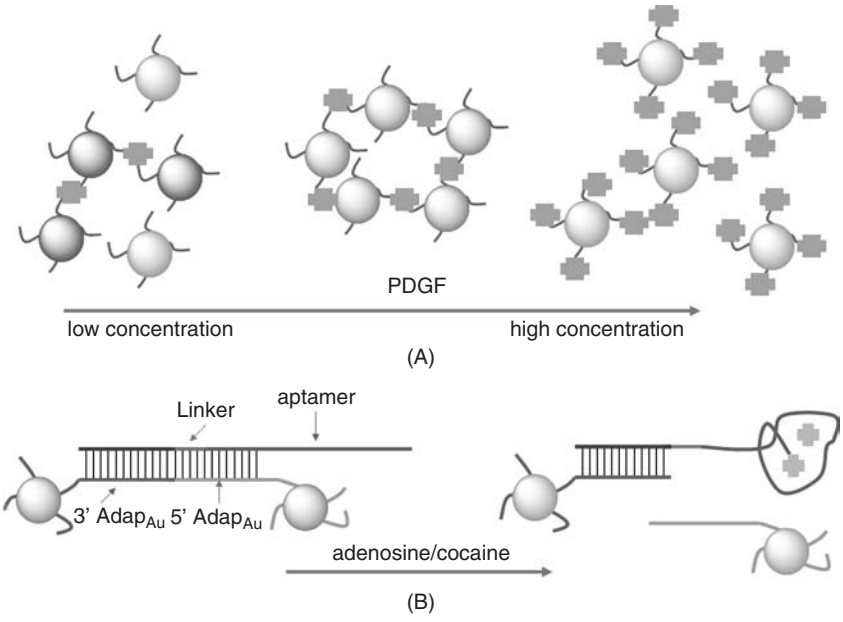

Figure 12.15 (A) Aggregation of aptamer-AuNPs in the presence of PDGFs at low, medium, and high concentrations; (B) colorimetric detection of small molecules. [(A) From Huang et al. (2005); (B) from Liu and Lu (2006).] (See insert for color representation.)

(Liu and Lu, 2006). In the presence of enough target molecules, the aggregates are destroyed by the competition between the target and the chain (5′-AdapAu), leading to a color change from purple to red. By this means, adenosine and cocaine are fast (less than 10 seconds) and easily detected with only the naked eye at some concentration range. "These aptamer-based tools could become a very useful addition to the nanostructure biodiagnostics work box. Imagine that simple litmus tests for every analyte for which aptamers exist were available!" (Famulok and Mayer, 2006).

In fact, such a duplex-to-complex concept is derived from a series of colorimetry processes developed for metal ions by the same group. Using the inherent properties of DNAzymes and a similar purple-to-red principle, metal ions such as Pb^{2+} (Liu and Lu, 2003), Cu^{2+} (Liu and Lu, 2007), and $UO_2{}^{2+}$ (Liu et al., 2007) have been detected efficiently. This duplex-to-complex concept is now being transferred to the "dipstick" method as a further step in approaching practical applications (Liu et al., 2006).

12.4.2 PFSFALF Mode

In fact, POSFALF colorimetric aptasensors described above are very simply fabricated; however, the PFSFALF aptasensors are designed more smartly and provide very interesting strategies. One of the most popular nonlabel routes in colorimetric aptasensnors still employs AuNPs as sensing elements. This is due primarily to the fact that the dispersed degree of the nanoparticles can be influenced readily by the surrounding environment, which may lead to a corresponding color change between red and purple.

In Rothberg's germinal work, they note the selective adsorption of ssDNA on AuNPs and show that ssDNA can stablize AuNPs against aggregation at a salt concentration that would ordinarily screen the repulsive interactions of citrate ions (Li and Rothberg, 2004). Because of that, the color of AuNPs is determined principally by surface plasma resonance, and because it is dramatically affected by nanoparticle aggregation, a simple colorimetric hybridization assay can be realized by using the difference between ssDNA and dsDNA electrostatic properties.

Inspired by Rothberg's work, nonlabeled colorimetric aptasensors have been fabricated. By making use of aptamers as nucleic acid molecules, this concept has been extended to additional targets, such as metal ions (Wang et al., 2006) and proteins (Wei et al., 2007)

Dong's group reports a simple, sensitive, and label-free 17E DNAzyme-based sensor for Pb^{2+} detection. The catalytic activity of some DNAzymes is divalent metal ion–specific, just as the catalytic activity of some protein enzymes is metal ion cofactor–dependent (Santoro and Joyce, 1997; Carmi et al., 1998). In this work, the authors focus on 17E DNAzyme, which is a divalent Pb^{2+}-specific enzyme employed widely in Pb^{2+} sensors (Santoro and Joyce, 1997). As shown in Figure 12.16A, in the presence of Pb^{2+}, 17E DNAzyme could cleave the substrate 17DS, which could release ssDNA (including 17E and fragments from 17DS)

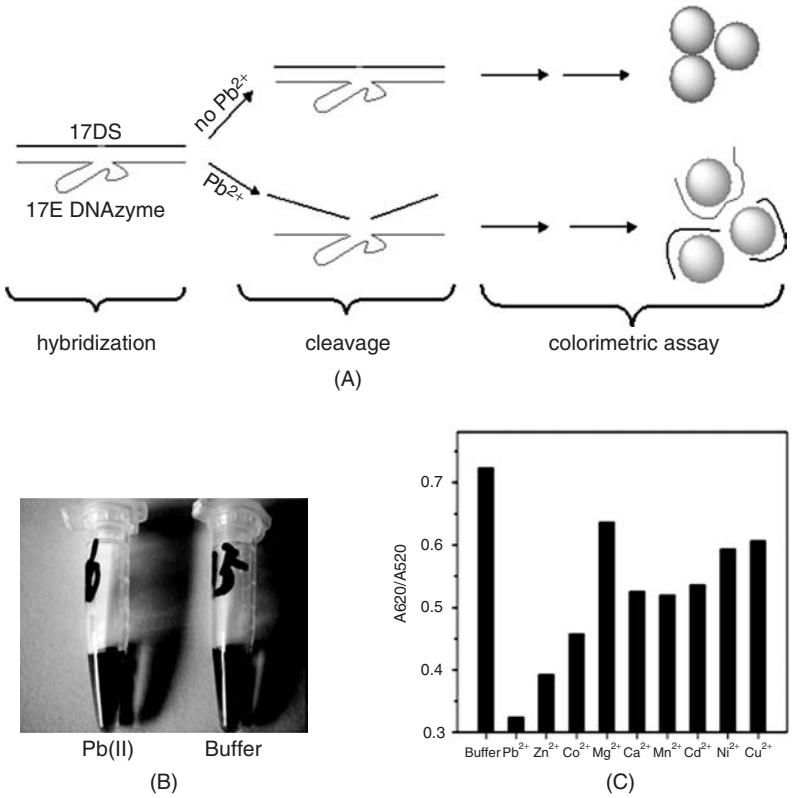

Figure 12.16 (A) AuNP-based colorimetric detection for Pb^{2+}. (B) 200 μL AuNPs/17E-17DS duplex solutions in the absence (right) and in the presence (left) of 50 μM Pb^{2+} after addition of 20 μL of 0.5 M NaCl. (C) Corresponding columns of the absorption ratio (A_{620}/A_{520}) of 200 μL of GNPs/17E-17DS duplex solutions in the presence of various 50 μM metal ions after addition of 20 μL of 0.5 M NaCl. [From Wei et al. (2008), with permission.] (See insert for color representation.)

from the hybridized 17E–17DS duplex. These ssDNA could be adsorbed on the red dispersed AuNPs, thus protecting the nanoparticles from aggregation in the presence of a given high concentration of salt (ca. 50 mM here). The characteristic SPR absorption band of AuNPs at about 530 nm is observed. However, the 17E–17DS duplex is retained in the absence of Pb^{2+}, which could not adsorb onto the AuNPs and thus could not stabilize the AuNPs under the same salt concentration. Accordingly, a shoulder band occurs at about 620 nm beside the SPR band at about 530 nm. When the Pb^{2+} is 50 μM, the color of AuNPs is changed from red to purple, which indicates that the Pb^{2+} can be monitored directly by the naked eye at this concentration (Figure 12.16B). Meanwhile, the absorption ratio (A_{620}/A_{520}) decreases gradually with an increase in Pb^{2+}

concentrations, presenting a positive correlation between decrease and Pb^{2+}. The selectivity of the sensors has already been proven through controlled experiments (Figure 12.16C). At a concentration of 50 μM, none of the other six metal ions, containing Mg^{2+}, Ca^{2+} Mn^{2+}, Cd^{2+}, Ni^{2+}, and Cu^{2+}, could elicit a response, which keeps the solution red. While in the absorption ratio, Zn^{2+} and Co^{2+} can respond slightly, which is in agreement with previous work (Brown et al., 2003).

Similar work does not depend on AuNP-based discriminability between ssDNA and dsDNA but on the significant structural variations of the aptamer upon binding with a metal ion (Wang et al., 2006). A DNA aptamer for K^+ that has the sequence 5′–GGG TTA GGG TTA GGG TTA GGG–3′ is employed as the model system in this work. The K^+ aptamer is a G-rich ssDNA and is random-coil-like in solution. Upon binding to its target (K^+), the aptamer folds to a four-stranded tetraplex structure (G-quartet) via intramolecular hydrogen bonds between guanines. It is found that like dsDNA, a G-quartet structure so formed still loses the ability to protect AuNPs from a high salt concentration (74 mM here) and results in a salt-induced aggregation of nanoparticles. The reason proposed is that on the one hand, DNA bases possess a high affinity to gold via coordination between the gold and nitrogen atoms (favoring DNA adsorption); on the other hand, negatively charged surfaces of AuNPs repel DNA phosphate backbones (disfavoring DNA adsorption) electrostatically. Such a G-quartet structure increases the surface charge density compared to unstructured ssDNA, and prevents the exposure of DNA bases to AuNPs, thus disfavoring adsorption of G-quartets on AuNPs in both facets. Unstructured ssDNA is soft and random-coil-like, which is in sharp contrast to the rigid structure of G-quartets. Therefore they possess greater freedom than G-quartets to wrap AuNPs. It may also contribute to the differentiation ability of AuNPs. So when the aptamer-AuNP solution is treated with increased K^+, an increased absorption ratio (A_{620}/A_{520}) is measured, due to the formation of increased G-quartets. At a concentration of 1.67 mM, an obvious color change from red to purple can be observed by the naked eye. These colormetric aptasensors were shown to be selective, for control sequences for both aptamers and other ions for K^+ do not respond.

Rationally, this method is applicable to most aptamers that undergo similar structural variations, from random coil to G-quartets, upon binding with targets. In another work, α-thrombin and its 29-mer aptamer are chosen as a model. The advantage of using a 29-mer prior to a 15-mer is evident (Wei et al., 2007). This 29-mer aptamer folds into a structure of G-quadruplex/duplex, which does not depend on the existence of K^+, an ion that stabilizes the G-quadruplex in a 15-mer aptamer. Therefore, the disturbance of K^+ (as described by Wei et al.) in the binding buffer can be neglected. Meanwhile, a 4-mer duplex formed together with a G-quadruplex (as shown in Figure 12.17A) makes the structure more stable while playing an important role in further repelling AuNPs as it does other ssDNAs.

In fact, as described by Wei et al., there is always equilibrium (for a 29-mer aptamer) between a random conformation and a G-quadruplex/duplex, whether or

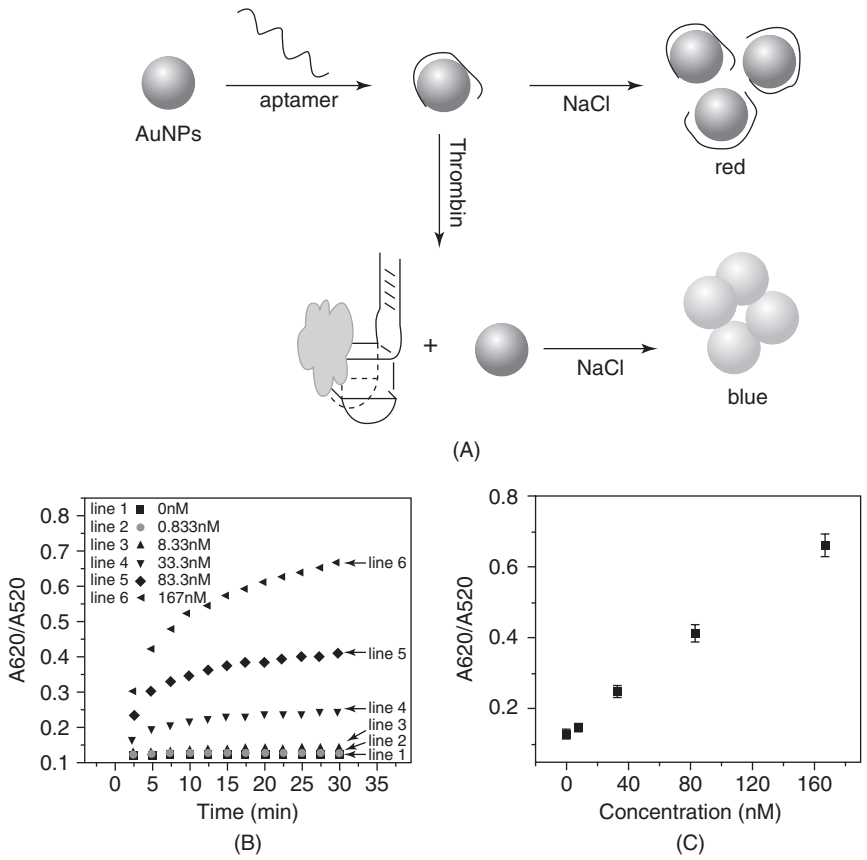

Figure 12.17 (A) AuNP-based colorimetric detection for α-thrombin. (B) 200 μL of AuNPs/17E-17DS duplex solutions in the absence (right) and in the presence (light) of 50 μM Pb^{2+} after addition of 20 μL of 0.5 M NaCl. (C) Corresponding columns of the absorption ratio (A_{620}/A_{520}) of 200 μL of GNPs/17E-17DS duplex solutions in the presence of 50 μM different metal ions after addition of 20 μL of 0.5 M NaCl. [From Wei et al. (2007), with permission. Copyright © 2007 Royal Society of Chemistry.] (See insert for color representation.)

not α-thrombin exists. But α-thrombin is favorable for this G-quadruplex/duplex conformation, so even through unfolded aptamer exists, the addition of α-thrombin would induce more aptamers to fold into a G-quadruplex/duplex conformation, which releases the AuNPs from being protected by ssDNA in a high salt concentration (166 mM NaCl). In this way, α-thrombin could be seen by the naked eye to detect selectively and sensitively in a concentration as low as 83 nM, and a linear range of 0 to 167 nM (as shown in Figure 12.17B and C) is obtained with a detection limit of 0.83 nM. Until this work, such smartly fabricated AuNPs-based colorimetric sensors had not been available for wider

applications. The method can also be used in systems in which an aptamer changes in structures without AuNP stability after binding.

However, "refinement" brings "default." Because a lot of analytes could maintain or destroy the stability of AuNPs themselves, the AuNP-based methods depend heavily on the nature of targets. In addition, if the samples themselves are colored, direct observation by the naked eye may be invalid. Therefore, this method may now be applicable only for simply composed samples. Much improvement is required before more complicated systems, especially biological samples, can be used.

In addition to AuNPs, other complexes are used in simple colorimetric aptasensors. In 2002, Stojanovic and Landry developed another nonlabel method, following their earlier work on fluorescent aptasensors. After a smarter route was designed using a colorimetric strategy. The authors tried to choose an aptamer that would bind both a chromophore and an analyte of interest. Therefore, the binding of analyte may alter the microenviroment of the chromophore and produce a visible signal of that event. Cocaine and its aptamer were chosen as a model, with cocaine binding the aptamer via a hydrophobic pocket formed by a noncanonical three-way junction with one of the stems structured through non-Watson–Crick interactions. The authors screened a collection of 35 dyes for changes in visible spectra upon addition of a stock solution of cocaine to a mixture of a given dye and the aptamer. One of the cyanine dyes, diethythiotricarbocyanine iodide, was finally chosen, for it could bind the aptamer, and even through the concentration of cocaine is as low as in the micromolar range, it could still be replaced by the analyte and display both a significant attenuation of absorbance and a change in the ratio of two relative maxima (750 nm for the monomer and 670 nm for the dimer) that dominated the visible spectrum. The sensing process follows the path shown in Figure 12.18A. An optimized aptamer/dye ratio is mixed in the binding buffer, and after equilibration for a given time, cocaine is added little by little, followed by acquisition of the absorption spectra within 1 minute. It is found that at a dye/aptamer ratio of 7 : 4 from 2 to 600 μM cocaine could be detected through decreased absorption at 760 nm, whereas at a higher dye/aptamer ratio, 0.5 to 67 μM cocaine could be detected through both decreased absorptions at 760 nm and increased absorptions at 670 nm. The detection is selective, for control molecules such as cocaine metabolites show no response. The sensing principle seems complex but is well explained in the paper. It is proved that there are two maxima for the dye, due to binding to DNA molecules as monomer (760 nm) and as dimer (670 nm). While in this system, the aptamer inclines more to bind the dimer. In the presence of cocaine, the analyte first interacts rapidly with the monometric dye–aptamer complex to release the dye, which results in the reduction of absorbance at 760 nm within seconds. The increase in absorption at 670 nm at the higher concentrations of the dye and aptamers is due to competition with cocaine of the dye released for binding to the remaining monometric dye–aptamer complex. In fact, a solution of the dye–aptamer complex displays a blue color. When cocaine is added, the solution would be decolorized after 12 hours, due to the appearance of a blue precipitate from hydrolysis of the dye

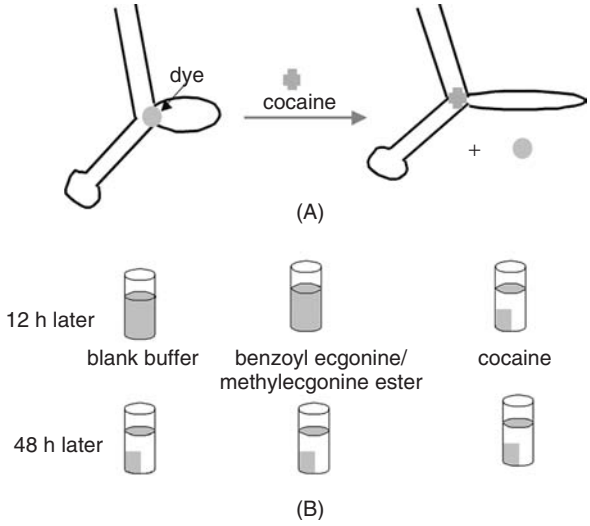

Figure 12.18 (A) Colorimetric method. (B) Mimic color of a dye–aptamer complex in the presence of: nothing, benzoyl ecgonine/ecgonine methyl ester, and cocaine, from left to right. [From Stojanovic and Landry (2002).]

in a slightly basic buffer, whereas for the solution itself or for added cocaine metabolites, this decolorizing reaction would happen after 48 hours. The difference between these times makes the visible colorimetric recognition applicable (Figure 12.18B). According to the authors, although the low-micromolar dissociation constant and selectivity for cocaine of this aptamer does not allow the determination of picomolar concentrations of cocaine metabolites in urine, the method is sufficient for handheld colorimetric field tests used in drug interdiction (Stojanovic and Landry, 2002).

New materials are still taken as recognition elements. A cationic, water-soluble, electroactive, photoactive polymer [one of the poly(3-alkoxy-4-methylthiophene)s] has been synthesized and used in protein detection (Ho and Leclerc, 2004). This polymer can exhibit chromic properties due to the conformational changes in the flexible conjugated backbone. When it is complexed to ssDNA or dsDNA, it displays important optical changes that can be used to transduce binding of an aptamer to a given target. Although an aqueous solution of polymer is yellow (with maximum absorption at 402 nm), a red color (with maximun absorption at 527 nm) is observed in the presence of ssDNA (X1–5′–GGTTGGTGGTTGG–3′ is used). This red shift is supposed to relate to a stoichiometric complexation between unfolded anionic ssDNA and the cationic polymer, and such polyelectrolyte complexes tend to be insoluble in the medium in which they are formed. The optical properties are different when K^+ (KCl) is present. This cation could facilitate the formation of a quadruplex state of X1 and stabilize it. In this case, polymer is allowed

to wrap this folded structure through electrostatic interactions, leading to an orange color (obviously, a blue shift in maximal absorption). Similar results are obtained when the chloride counterion is replaced by a bromide or iodide anion, indicating the selectivity of detection toward K^+.

X1 is also the 15-mer aptamer for α-thrombin, so this route is applicable for α-thrombin detection too. In the presence of K^+, the quadruplex state of X1 is also promoted by α-thrombin binding. Accordingly, for the 1 : 1 : 1 complex between polymer, X1, and α-thrombin, an orange color is observed, with the same absorption spectrum as that induced by K^+. In this way, α-thrombin can be recognized directly by observing the color of the sample solution. However, such detection has a relatively poor detection limit of 1×10^{-7} M, which is much higher than most reported α-thrombin sensors (most have a detection limit no higher than about the nanomolar range. Interestingly, when taking the fluorescent properties of polymer instead, very small quantities of α-thrombin can be detected in aqueous solutions. The fluorescence of the polymer is quenched in the presence of random-coil ssDNA (X1), whereas when the DNA is in a quadruplex state by binding α-thrombin, the yellow form of the polymer is fluorescent (the maximum emission at 525 nm). Detection is sensitive, under these conditions, as 1×10^{-11} M α-thrombin can be detected, which is 10,000-fold more sensitive than can be achieved using the UV–visible spectrum. This work is shown schematically in Figure 12.19.

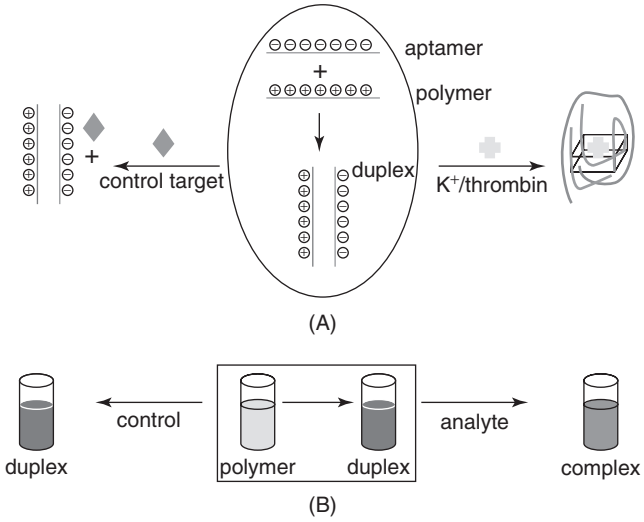

Figure 12.19 (A) Principle of specific detection. (B) Mimic colorimetric recognition of analytes such as K^+ and α-thrombin. It is observed that after interacting with DNA, the color of the polymer is changed from yellow to red. If analyte is added, the color is blue-shifted. However, if control molecules such as LiCl/BSA are added, no color change can be observed. [From Ho and Leclerc (2004).]

Another type of widely used colorimetric aptasensor employs hemin and its aptamer as a DNAzyme complex. Since the DNAzyme complex catalyzes chemical reactions to produce colored products, which is different from the colorimetric detection described above, we will discuss this type of aptasensor in a separate section.

12.5 HEMIN–APTAMER DNAzyme-BASED APTASENSOR

The DNAzyme itself is used as a biocatalyst. Another interesting example of a catalytic DNA is that of certain peroxidase-like activities, including a supramolecular complex between hemin and a single-stranded guanine-rich nucleic acid aptamer (Travascio et al., 1998). It is suggested that the supramolecular docking of the guanine–quadruplex layers facilitates the intercalation of hemin into the complex and significantly enhances the biocatalytical activity of the hemin center. Using these peroxidase-like activities, another type of colorimetric aptasensor in PFSFALF can be developed. With nothing labeled, the sensing processes proceed smartly.

Willner's group has been engaged in applying this method to DNA sensing and has made many improvements (Xiao et al., 2004; Yi et al., 2004). Usually, they have employed a peroxidase-like catalytic reaction in which hemin–aptamer complex catalyzes the reaction oxidation of 2,2′-azino-bis(3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) by H_2O_2 (Xiao et al., 2004). The product ($ABTS^+$) is a colored molecule that can transfer signals directly by its absorbance at 414 nm.

Figure 12.20 depicts one of the designs of Willner's group. A molecular beacon ssDNA is synthesized in a hairpin structure consisting of two parts. One is a template segment that is complementary to the target ssDNA. The other is an antihemin aptamer, part of which is complementary to the template part in forming a duplex in the hairpin. At this conformation, even in the presence of hemin, the formation of catalytic DNAzyme is prohibited. Once a fully complementary target DNA exists, it will hybridize with the template part and release the hemin aptamer to a completely free configuration. Then a catalytic DNAzyme can be formed and oxidation of the ABTS by H_2O_2 can be realized. The result can be read out through $ABTS^+$ accumulation at 414 nm. Although hemin itself also possesses catalytic activity, it is proven that at the pH value used, 8.1, this activity is too poor to lead to an effective oxidation reaction. It indicates that a detectable signal results primarily from the catalytic activity of DNAzyme, with no disturbance from hemin itself. In this way, a detection limit of 8 μM is reached. Meanwhile, it is found that the response of a completely complementary target (for the template part) is about eightfold higher than even that of a single-mismatched target, which exhibits the ability of the sensor to discriminate among gene mutants. The method was later improved by combining it with PCR (Xiao et al., 2004). Although the experiments are more complex, much higher sensitivity is achieved. Forty molecules could be detected in a 50-μL sample, which displayed an ultrasensitive detection.

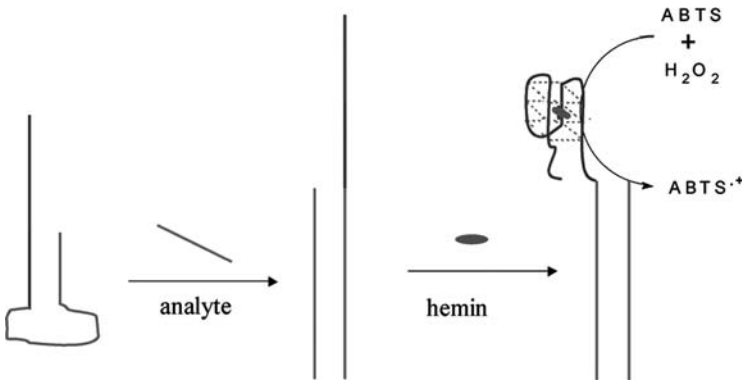

Figure 12.20 Analysis of DNA by opening of a beacon nucleic acid and the generation of DNAzyme. [From Xiao et al. (2004).]

Dong's group has adopted a different DNAzyme-based strategy for DNA sensing (Li et al., 2007d). As shown in Figure 12.21A, the antihemin aptamer is divided into two separate parts (S1 and S2), with a template ssDNA tail on each end. In the presence of hemin, the two parts self-assemble on the hemin site to form a layered G-quadruple supramolecular complex (DNAzyme, $K_d \sim 130$ nm). At a pH of 8.0, this DNAzyme can catalyze the oxidation of ABTS by H_2O_2, and the disturbance from hemin itself can still be neglected. The authors adopted two separate sensing processes, adding hemin either following or preceding the target (S3) and then comparing different results observed. In the former process, similar to earlier work (Yi et al., 2004), DNAzyme is not formed when the template part has hybridized with the target. Therefore, it is a signal-off process in which the more target that is added, the lower the signal that is produced. However, the opposite results appear when the hemin is added before the target. The authors hypothesize that the supramolecular complex here is not as stable as the hemin/G-quartet structure formed directly by hemin and its 18-mer DNA aptamer due to its ternary structure and two free tails. However, in the presence of target DNA, the two parts (of the supramolecular complex) are attached further through part hybridization (Figure 12.21A) and become more stable. Thus, the catalytic activity is enhanced, leading to a signal-on process. It should be noted that in this condition, the target DNA is not entirely hybridized with the template DNA, due to the block coming from the supramolecular complex formed. Therefore, this signal-on phenomenon is applicable only for targets at low concentration, ranging from 0.005 to 0.3 μM ($r = 0.997$) (Figure 12.21B). When the concentration is increased further, the signal decreases. This may be attributed to the fact that more and more targets begin to bind with the template parts in an entirely hybridized conformation, which blocks the formation of a supramolecular complex, just as in the process in which hemin is added after the target. So as shown in Figure 12.21B, when the concentration ranges from 0.3 to 1.0 μM, the signal

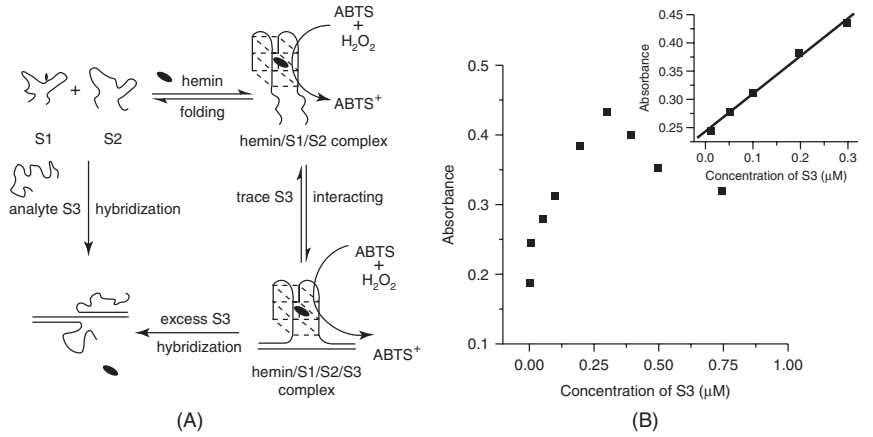

Figure 12.21 (A) Schematic of label-free colorimetric approaches for sensing DNA by using a supramolecular hemin/G-quartetcomplex with two free nucleic acid parts as the sensing element (template DNA). The DNAzyme is formed by incubating hemin with two guanine-rich single-strand DNAs (S1, S2). The complementary DNA (S3) is used as the analyte. (B) Investigation of the effect of S3 on the catalytic activity of the hemin/S1/S2 complex by using ABTS/H_2O_2 colorimetric detection. The figure represents the dependence of the absorbance of $ABTS^+$ on the S3 concentration. The insert reveals a linear relation between the absorbance and S3 concentration. [From Li et al. (2007d), with permission. Copyright © 2007 Royal Society of Chemistry.]

is gradually decreased. Obviously, the signal-on process is more sensitive, but the detection range is limited.

Recently, such hemin–aptamer DNAzyme-based sensors have been used for with more application binding with aptamers of other analytes. Taking lysozyme detection, for example, a part duplex is first hybridized from two elaborately designed DNA strands (Di et al., 2007). One is a sensing sequence containing a region of lysozyme aptamer and another of hemin aptamer. The second is a blocking sequence, used to include two separate nine-base complementary domains in a sensing sequence, resulting in cooperative binding. With hemin but no analyte (lysozyme here), formation of the DNAzyme is seriously inhibited by the blocking sequence, and low catalytic activity can be tested. If lysozyme is added, the affinity coming from both lysozyme–aptamer and hemin–aptamer will obviously reduce the melt temperature of the duplex, which finally leads to dehybrization of the duplex and the formation of two aptamer–substrate complexes. Correspondingly, the DNAzyme will display its catalytic activity. In this work, an ABTS–H_2O_2 detection system is adopted, so the signal can be read out directly from the increased absorbance at 414 nm, and a detection limit of 1×10^{-13} M is achieved, which has been one of the most sensitive detections for this analyte until now. Meanwhile, small-molecule AMP is detected in the same way (detection limit: 4×10^{-6} M), indicating the universality of this strategy.

12.6 LIQUID CHROMATOGRAPHY, ELECTROCHROMATOGRAPHY, AND CAPILLARY ELECTROPHORESIS APPLICATIONS

Aptamers have also been employed in various chromatography techniques, such as liquid chromatography (Romig et al., 1999), electrochromatograpy (Connor and McGown, 2006), and capillary electrophoresis (Berezovski and Krylov, 2005). The advantages of aptamers applied in this field are obvious: Wide targets enlarge the range of analytes that can be used; easy synthesis reduces the cost; selected as functional oligonucleotides decreases the adsorption (compared with antibodies); and high affinity guarantees a high selectivity and separation effect. Based on these advantages, processes such as molecular recognition (Cho et al., 2004; Li et al., 2007a, 2007c), quantitative detection (German et al., 1998; Pavski and Le, 2001), analogy separation (Deng et al., 2001), and even chiral selection (Michaud et al., 2003; Brumbt et al., 2005) have been undertaken using aptamer-based chromatography analysis. Chromatography techniques are also used to assist the SELEX procedure and exhibit increasing potential in this field.

Some analytical applications in chromatography using apamers are summarized in Table 12.3. One can see the rapid development of the use of aptamers from the table. However, most aptamer-based chromatography techniques are not PFSFALF. First, for example, if the aptamers are used to enhance the separation effect, they usually need to be modified onto the inner walls of the column (Connor and McGown, 2006). Second, due to relatively limited detection methods coupled with chromatography (e.g., fluorescence), even when aptamers are used as recognition and seperation materiels simply by being added to running buffers or sample solutions, the aptamers or targets themselves still need to be derived or labeled to produce detectable signals (German et al., 1998). Even so, there is still a chance to make PFSFALF available in aptamer-based chromatography techniques. That is what we focus on in this section. In the following descriptions, we give several examples of the use of the CE technique. Most chromatography techniques possess different operational principles but similar functions, such as separation.

Recently, Dong's group used analytes as indicators of themselves and has developed a series of selective aptamer-based PFSFALF coupling CE separations using different detection methods (T. Li et al., 2007a). A small molecule of cocaine is first taken as a model molecule to be recognized and separated from its analogs (T. Li et al., 2007a). Both cocaine and its analog, ecgonine, contain a tertiary amino group and can generate strong electrochemiluminesence (ECL) emission on a platinum electrode in the presence of tris(2,2′-bipyridyl)ruthenium(II), $[Ru(bpy)_3]^{2+}$. So when the two analogs are mixed and separated through CE, two peaks of ECL appear (Figure 12.22A). Once cocaine-binding aptamers are added to the sample, the net amount of cocaine will be reduced, due to the formation of an aptamer–cocaine complex. Following CE, such a complex can be separated from the surplus cocaine as well, which ultimately leads to a reduced cocaine peak. By contrast, the ecgonine is not affected by the aptamer and

TABLE 12.3 Aptamers in Chromatography Techniques

Target	Oligo-nucleotide	Separation System[a]	Detection System[a]	Model	Ref.
L-Selectin	DNA	LC	UV	PFSOALF	Romig et al., 1999
α-Thrombin	DNA	CEC	FL	PFSOALF	Connor et al., 2006
HCVRNA polymerase	RNA	LC/chip	MALDI-TOF	PFSOALF	Cho et al., 2004
HCVRNA replicase	RNA	LC/chip	FL	PFSOALF	Chung et al., 2005
Adenosine and analogs	DNA	Nano-LC	UV	PFSOALF	Deng et al., 2001, 2003
Cocaine and analogs	DNA	CE	ECL	PFSFALF	Li, T. et al., 2007a
Amino acid amides	DNA	CE	EC	PFSFALF	Li, T. et al., 2007c
HRP and hemin	DNA	CE	CL	PFSFALF	Li, T. et al., 2007b
FMN and FAD	RNA	CEC	UV	PFSOALF	Clark et al., 2003a
FMN and thiourea	RNA	CEC	UV	PFSOALF	Clark et al., 2003b
IgE and α-thrombin	DNA	CEC	LIF	POSFALF	German et al., 1998; Buchanan et al., 2003
HIV-1 RTase	DNA	CEC	LIF	POSFALF	Pavski et al., 2001; Wang, H. L. et al., 2005; Fu et al., 2006
α-Thrombin and anti-α-thrombin III	DNA	ACE	LIF	POSFALF	Huang et al., 2004
α-Thrombin	DNA	ACE	LIF	POSFALF	Berezovski et al., 2003
Taq DNA polymerase	DNA	ACE	LIF	POSFALF	Berezovski et al., 2005
Nontarget proteins					
β-Lactoglobulin A and B	G-quartet DNA	CEC	UV	PFSOALF	Rehder et al., 2001
Bovine milk proteins	G-quartet DNA	CEC	UV	PFSOALF	Rehder-Silinski et al., 2003
Albumins	G-quartet DNA	CEC	UV	PFSOALF	Dick et al., 2004

(*continued*)

TABLE 12.3 (*Continued*)

Target	Oligo-nucleotide	Separation System[a]	Detection System[a]	Model	Ref.
Nontarget species					
Binary mixtures of amino acids(D-trp and D-tyr), enantiomers (D-trp and L-trp), and polycyclic aromatic hydrocarbons	G-quartet DNA	CEC	UV	PFSOALF	Kotia et al., 2000
Isomeric dipeptides Trp-Arg and Arg-Trp	G-quartet DNA	CEC	UV	PFSOALF	Charles et al., 2002
Homodipeptides and alanyl dipeptides	G-quartet DNA	CEC	UV	PFSOALF	Vo et al., 2004
Fibrinogen peptides	G-quartet DNA	CEC	UV	PFSOALF	Vo et al., 2006
Enantiomers					
Arginine-vasopressin	DNA	LC	UV	PFSOALF	Michaud et al., 2003
Adenosine	DNA	Micro-LC	UV	PFSOALF	Michaud et al., 2004
Tyrosinamide	DNA	Micro-LC	UV	PFSOALF	Michaud et al., 2004
Arginine	RNA	Micro-LC	UV	PFSOALF	Brumbt et al., 2005
Amino acids and derivatives	RNA	Micro-LC	UV	PFSOALF	Ravelet et al., 2005

[a] **ACE**, affinity capillary electrophoresis; **CEC**, capillary electrochromatography; **CL**, chemiluminescence; **EC**, electrochemistry; **ECL**, electrochemiluminescence; **FAD**, flavin adenine dinucleotide; **FMN**, flavin mononucleotide; **HCV**, hepatitis C virus; **HIV-1 RTase**, reverse transcriptase of human immunodeficiency virus type 1; **LC**, liquid chromatrography; **LIF**, laser-induced fluorescence; **MALDI-TOF**, matrix-assisted laser desorption ionization–time-of-flight mass spectrometry; **UV**, UV–visible spectrum.

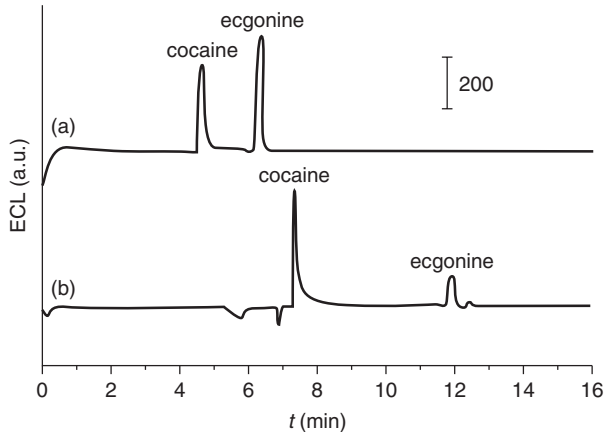

Figure 12.22 CE-ECL analyses for a mixture of 600 mM cocaine and 400 mM ecgonine using 20 mM phosphate (pH 7.0) in the (a) absence and (b) presence of 1% $BMIMBF_4$ running buffer. The upright bar refers to an ECL intensity of 200 (a.u.). [From T. Li et al. (2007a), with permission. Copyright © 2007 Wiley.]

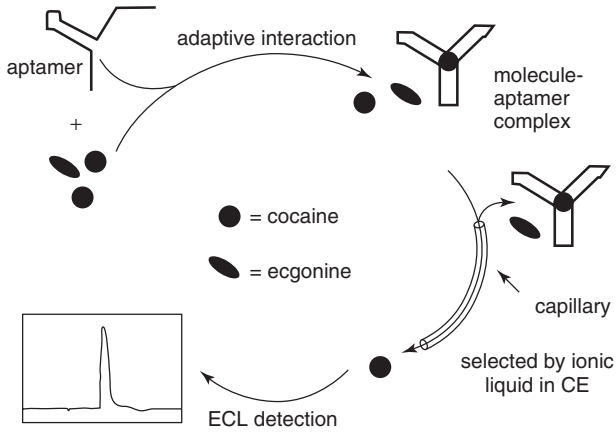

Figure 12.23 Label-free method for aptamer-based recognition of cocaine from its hydrolysate (ecgonine) using CE-ECL analysis assisted by an ionic liquid selector. An ionic liquid was used as the selector for the sample injection in the CE process, enriching cocaine and excluding the cocaine–aptamer complex and ecgonine from the capillary. [From T. Li et al. (2007a), with permission. Copyright © 2007 Wiley.]

keeps its peak without change (Figure 12.23). What should be noted is that no peak of aptamer–cocaine complex is observed. The explanation for this phenomenon is as follows: First, interaction between the aptamer and redox-active sites on the cocaine molecules could be taken up through aptamer binding, which results in disappearance of the ECL emission. This phenomenon may

be applicable for many other molecules as well. Second, in the running buffer, 1% room-temperature ionic liquid [1-butyl-3-methylimidazolium tetrafluoroborate ($BMIMBF_4$)] is added to serve as a selector during the sample injection process. As in Dong's group's earlier report, when a selector is used, positive molecules such as cocaine (pH 7.0) can be enriched during sample injection; while a negative molecule of ecgonine (pH 7.0), aptamer, and cocaine–aptamer complex will be repelled out of the capillary to a large degree (as in Figure 12.24). So in this system no cocaine–aptamer complex peak is observed and the adsorption of DNA onto the capillary inner wall is further reduced. At the same time, the ecgonine peak is greatly reduced (Figure 12.22B). Through the peak decrease, cocaine was sensitively recognized using itself as a signal probe in the presence of its analog and a detection limit as low as 2 μM. This method has also been proven feasible in a biological system of 25% fetal bovine serum.

The advantages of this method are obvious. First, taking analytes themselves as indicators binding with a CE separation technique, all possible label processes were avoided, which makes recognition very simple and convenient. Second, aptamers as recognition elements could be a very useful alternative to the traditional spike method, especially when peaks of analytes are overlapped to a large degree with other coexisting materials. Here just depending on whether the peak is lowered in the presence of aptamers, we could judge the existence of the target and even its exact amount. Finally, but no less important, a lot of molecules may produce detectable signal according to their intrinsic properties. The method has a promising potential for wider targets using various detection techniques, such as electrochemistry, chemiluminescence, UV, and LIF.

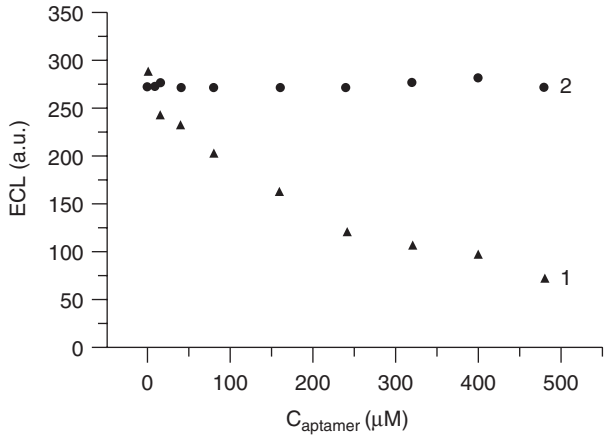

Figure 12.24 Dependence of ECL intensities of cocaine and its analog on the concentrations of aptamer A1 after incubation for 1 hour at room temperature in 20 mM phosphate of pH 7.0. (1) 800 mM cocaine, (2) 1.3 mM ecgonine. [From T. Li et al. (2007a), with permission. Copyright © 2007 Wiley.]

In recent work using a similar principle, the amino acid amides argininamide (Arm) and tyrosinamide (Tym) were recognized successfully from their analogs as well, using CE electrochemical detection (T. Li et al., 2007c). Then the some hypothesis is made regarding conformation of the Tym-binding aptamer. Many guanines are spaced in a regular pattern as a G-doublet in the consensus sequence of the Tym aptamer used, so it is naturally hypothesized that it may fold into a G-quartet structure for binding Tym. However, no signal differences are found when the binding reaction is carried out in a buffer with 20 mM K^+. It is well known that the G-quartet structure depends strongly on the existence of K^+, so no effect by K^+ on binding affinity may lead to the hypothesis that no G-quartet formed in this process.

Another work focuses on a small molecule of hemin with CE chemiluminescence detection and has been extended to aptamer-based DNA assays (T. Li et al., 2007b). Both hemin and a hemin-18-mer/aptamer complex (DNAzyme) can catalyze the chemiluminescent generation of luminol in the presence of H_2O_2. Coupled with CE separation, hemin is first sensitively distinguished from the control molecule HRP at pH 11. Under these conditions, the catalytic activity of hemin is optimized but that of synthetic DNAzyme is seriously inhibited, which reduces signal interference for the entire system. This property is then applied to the detection of target ssDNAs that could form G-quartets with the templet ssDNA (Figure 12.25). In this case, both hemin and templet are in excess. In the presence of target DNA, the templet DNA composes to form a G-quartet that could bind with hemin. Thus, the free hemin is reduced. Following CE, free hemin is well separated from the target–templet–hemin

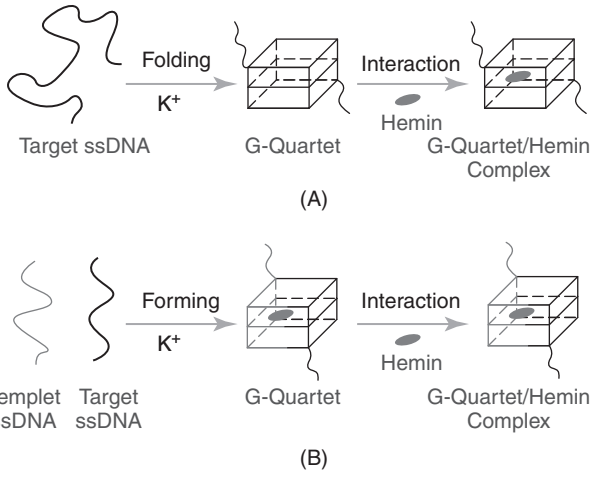

Figure 12.25 Label-free approach to DNA detection using CE-CL analysis: (A) Detection of ssDNAs that can fold into the G-quartet structure; (B) Detection of ssDNAs that can form the G-quartets with the templet ssDNA. [From T. Li et al. (2007b), with permission.]

complex and the a decreased CL signal is observed for the free hemin. In this way, ssDNA is quantitively detected even at conditions under which both hemin and target–templet–hemin complex will produce a CL signal. The detection limit of ssDNA is 0.1 μM. Although it is not as sensitive as many other DNA sensors, it does provide a smart way to achieve label-free DNA detection using the CE technique and shows promising potential for future study.

12.7 OTHER APTASENSORS

In addition to the methods described above, other techniques, such as ion-selective field-effect transistors (ISFETs) (Zayats et al., 2006), quartz crystal microbalance (QCM) (Tombelli et al., 2005b), love waves (Schlensog et al., 2004), FM (Basnar et al., 2006), and SPR (Balamurugan et al., 2006), are also used for aptasensor fabrication. Convenient and smart aptamer-based detections are easily realized with these techniques because that all of them depend directly on changes happening on the substrate surfaces to produce detectable signal: for example, charge change (and thereby potential change) in ISFETs and the mass change associated with crystals according to the Sauerbrey equation.

Using these techniques, both protein and small-molecule are successfully detected.

12.8 CONCLUSIONS

In this chapter we discuss mainly the development of easily fabricated aptasensors: label-free aptasensors. As novel functional nucleic acids, aptamers have displayed many advantages over traditional recognition elements, especially in simplifying the entire detection process. Thus, usually, such simple routes are fast, sensitive, and selective, and defaults must be considered. It is found that many of the easy routes are not applicable to practical detection, or rather, detection in complex biological conditions. Sensors used when no labeling is required, especially, sometimes seem to be too smartly designed to overcome disturbances in practical samples that contain various types of complexes. Therefore, more effort will be needed before these sensors can be used successfully in practical samples.

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