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Bioelectronics

Electronic Aptamer-Based Sensors

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The selection of aptamers—nucleic acids that specifically bind low-molecular-weight substrates or proteins—by the SELEX (systematic evolution of ligands by exponential enrichment) procedure has attracted recent efforts directed to the development of new specific recognition units. In particular, extensive activities have been directed to the application of aptamers as versatile materials for the design of biosensors. The Minireview summarizes the recent accomplishments in developing electronic aptamer-based sensors (aptasensors), which include electrochemical, field-effect transistor, and microgravimetric quartz crystal microbalance sensors, and describes methods to develop amplified aptasensor devices and label-free aptasensors.

1. Introduction

Aptamers are nucleic acids (DNA or RNA) that selectively bind to low-molecular-weight organic or inorganic substrates or to macromolecules such as proteins.[1] The affinity constant of aptamers towards their substrates lies in the micromolar to nanomolar range, comparable to the binding constants of antibodies to antigens.[2] The interest in aptamers as specific binding agents originates from the relative ease of their preparation by an evolutionary selection procedure that eliminates the need for sophisticated design of the receptor units. The selection of the aptamers for the specific target is based on the SELEX procedure (systematic evolution of ligands by exponential enrichment),[3] which is schematically explained in Figure 1. The process is initiated with a random library of oligonucleotides (usually 10^{15}–10^{16}), which consist of linear nucleic acids with a random sequence embraced by a 5′ and a 3′ nucleic acid sequence of defined constant composition. An RNA-searched aptamer involves the primary transcription of the DNA library into an RNA pool, followed by passing the library through a separating matrix that includes the target substrate. The few nucleic acids that reveal affinity towards the substrate (or some nonspecific nucleic acid adsorbents) bind to the separation matrix, while most of the library ingredients are washed off. The elution of the surface-bound nucleic acids followed by their PCR amplification yields a mixture of nucleic acids with variable affinity towards the target. Repeated separation of the mixture on the ligand-modified surface results in the enrichment of the mixture with the nucleic acids that reveal a high affinity for the substrate, and usually 8–15 repeated separation–amplification cycles yield the aptamers. The selection of DNA-based aptamers proceeds by an identical selection mechanism that eliminates the primary transcription step. The single-stranded DNA/

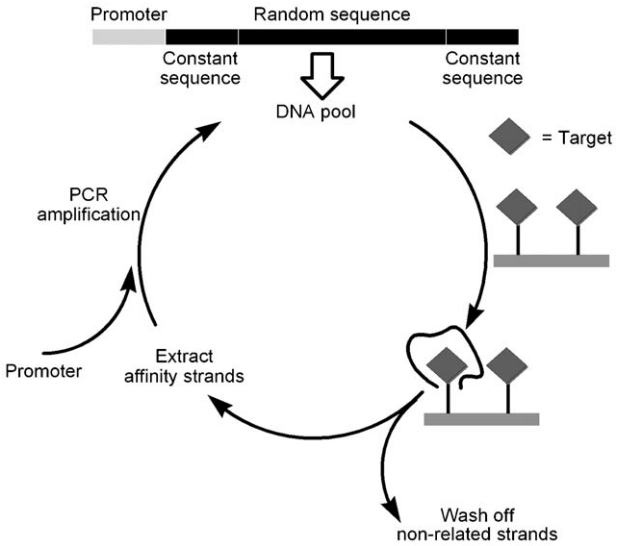

Figure 1. The preparation of an aptamer by the systematic evolution of ligands by exponential enrichment (SELEX) protocol. PCR: polymerase chain reaction.

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RNA aptamers associate to their targets by generating three-dimensional structures that involve the generation of loops and hybridized regions using complementary Watson–Crick base-pairing.[4] The final identification of the aptamer composition by sequencing and elucidation of the aptamer–substrate tertiary structure by NMR spectroscopy[5] leads then to the selection of a high-affinity binding material from a random composite. PCR amplification provides then an unlimited synthetic source for the aptamers.

Not surprisingly, aptamers have found growing interest as active separation materials in chromatography[6] and electrophoresis,[7] as therapeutic and diagnostic agents,[8] and as active materials for biosensing.[9] The use of aptamers for biosensing is particularly interesting, as aptamers could substitute antibodies in bioanalytical sensing and reveal obvious advantages over immunosensors: 1) The in vitro selection of the aptamers eliminates the need for the in vivo immunization of animals required to elicit antibodies. This enables the fabrication of aptamer binding ligands for toxic materials such as toxins that are impossible to obtain by the immune system. 2) In contrast to antibodies that undergo mutation and structural perturbation during the eliciting process, the chemical synthesis of aptamers usually leads to highly reproducible structures of the binding ligands. 3) The chemical modification of aptamers with optical or redox-active labels or with functional groups that enable their tethering to transducers is usually easier than the manipulation of antibodies. 4) The direct modification of the aptamers with imaging labels allows the direct readout of the aptamer–substrate complex, without the need for a complex antigen–antibody sandwich assay. This is particularly important for low-molecular-weight substrates, where immunosensors are subjected to competitive assays only. 5) Non-specific adsorption phenomena are usually less pronounced on nucleic acid interfaces as compared to protein interfaces. Also, the thermal stability of nucleic acids, as compared to antibodies, adds attractive advantages for using aptamers as the active sensing material—aptasensors.

Despite the list of advantages of aptamers as compared to antibodies, a fair comparison should highlight also the disadvantages: DNA and especially RNA are very sensitive to hydrolytic digestion by nucleases, thus requiring highly pure environments for their applications. Several solutions to transform aptamers into nuclease-resistant moieties by the modification of the ribose ring at the 2′-position[10] or by the specific modification of the pyrimidine nucleotide were reported.[11]

Indeed, numerous studies have reported on the development of optical aptasensors,[12,13] and the progress in the field has been summarized in several review articles.[9] We are, however, witnessing rapid developments in the design of electronic (electrochemical, field-effect transistor, and piezoelectric) aptasensor systems. This Minireview summarizes these accomplishments and highlights the advantages of electronic transduction of aptasensor systems.

2. Electrochemical Aptasensors

The tremendous advances in electronic biosensor design and, particularly, the development of amplification routes for detection schemes has allowed the adaptation of these concepts for the development and fabrication of aptasensors. Catalytic labels, such as enzymes, inorganic or organic catalysts, or nanoparticles, are often used for recognition processes. Enzymes, and specifically redox enzymes, are often coupled to antibodies or nucleic acids, and these act as biocatalytic conjugates for the amplified electronic readout of immunocomplexes or DNA analysis. For example, the hydrolysis of *p*-aminophenyl phosphate by an antibody–alkaline phosphatase conjugate generates *p*-aminophenol as an electrochemically detectable product.[14] The electrochemical analysis of DNA was accomplished by the use of nucleic acid functionalized horseradish peroxidase (HRP) as label for the bioelectrocatalyzed reduction of H_2O_2.[15] Similarly, the enzyme-catalyzed precipitation of an insoluble product on electrodes was used for the amplified electrochemical or microgravimetric detection of biorecognition events. For example, biotin-labeled HRP or alkaline phosphatase, or nucleic acid functionalized alkaline phosphatase, were used as labels for the analysis of immunocomplexes,[16] DNA,[17] or single-base mismatches in DNA[18] through the biocatalyzed precipitation of insoluble products on the electrode, such as an insoluble indigo product. They were also used for the detection of biorecognition events by either monitoring the insulation of the electrode using Faradaic impedance spectroscopy or by measuring the changes in mass on piezoelectric quartz crystals.

Two different configurations that employ the biocatalytic properties of enzymes to detect and amplify the analysis of

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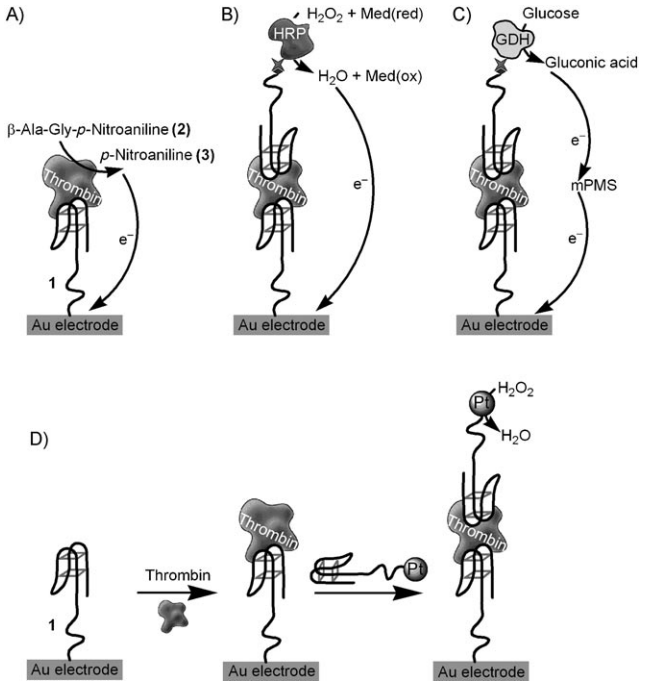

Figure 2. The amplified electrochemical analysis of thrombin. A) The thrombin-catalyzed generation of the electroactive substrate **3**. B, C) Enzyme-tethered anti-thrombin aptasensors as amplifying labels (horseradish peroxidase (HRP) and glucose dehydrogenase (GDH), respectively). Med: mediator; mPMS: 1-methoxy-5-methylphenazinium methyl sulfate. D) Pt-NP-labeled aptamers as electrocatalysts.

thrombin by its aptamer were reported (Figure 2).[19] In one approach (Figure 2A), the thiolated aptamer **1** was assembled on a Au electrode, and this assembly acted as a sensing interface for the association of thrombin. The bound thrombin acted as a protease and hydrolyzed a nitroaniline-functionalized peptide **2** to *p*-nitroaniline (**3**). The latter product was then analyzed electrochemically. The second configuration mode (Figure 2B) makes use of the fact that the thrombin includes two different binding sites for aptamers.[20] Accordingly, a sandwich-type assay, in which a second aptamer bound to HRP was linked to the primary aptamer–thrombin complex, was used as an amplifying reporter label in the system. The enzyme catalyzed the relay-mediated reduction of H_2O_2. The amperometric response was then used to quantify the concentration of thrombin, which could be analyzed by this method with a detection limit of 8×10^{-8} M.

Also, the pyrroloquinoline quinone dependent glucose dehydrogenase (GDH) was employed as a biocatalytic label for the amplified amperometric detection of thrombin (Figure 2C).[21] The fact that two different DNA aptamers bind to thrombin[20] was utilized to develop the aptasensor for thrombin. Thrombin was linked to a 15-mer thiolated aptamer linked to an Au electrode, and the GDH–avidin conjugate was linked to the surface by its association to the biotinylated 29-mer aptamer bound to the thrombin on the surface. The bioelectrocatalyzed oxidation of glucose in the presence of 1-methoxy-5-methylphenazinium methylsulfate (mPMS) as diffusional mediator enabled the amperometric detection of the thrombin with a linear response in the range of $4–10\times10^{-8}$ M.

A related approach substituted the biocatalysts with Pt nanoparticles (NPs) as catalytic labels (Figure 2D).[22] Pt NPs were functionalized with the thiolated aptamer **1**. The stepwise assembly of the aptamer **1** on the surface and the subsequent formation of the aptamer–thrombin complex on the surface were monitored by the association of the aptamer-functionalized Pt NPs to the aptamer–thrombin complex assembled on the electrode. The nanoparticles catalyzed the electrochemical reduction of H_2O_2, and the resulting cathodic currents enabled the amplified detection of thrombin with a detection limit of 1×10^{-9} M. This latter system is very similar to the biocatalytic sandwich-type analysis of thrombin using HRP or GDH as biocatalysts, (Figure 2B, C). Nonetheless, the Pt NP catalyst reveals an 80-fold enhanced limit of detection. The improved sensitivity observed with the nanoparticles may be attributed, in part, to the need to activate the biocatalytic functions of enzymes by a diffusional electron mediator that is weakly coupled with the electrode surface as a result of the aptamer layer and protein nonspecific adsorptions (which does not occur in the case of the nanoparticle system).

The conformational changes of nucleic acids upon hybridization have formed the basis for the development of different electrochemical DNA sensors.[23,24] For example, upon immobilization on a conducting support, a molecular hairpin-like DNA stem-loop structure labeled with a redox-active reporter reveals electron-transfer communication with the electrode, as a result of the close proximity of the redox label in the stem-loop structure to the electrode surface. However, hybridization of the stem-loop structure with a complementary DNA resulted in an extended duplex structure that positioned the redox label in a spatially separated configuration that prevented electrical contact with the electrode. This behavior enabled the amperometric detection of the DNA by following the perturbation of the electrical contacting of the redox label with the electrode upon hybridization.[23] Single-stranded nucleic acids that act as aptamers for proteins or small molecules change their flexible single-stranded chains into well-defined 3D structures upon complexation with their host substrates. This behavior enabled the tethering of redox-active units to the aptamer nucleic acids and the identification of the formation of the aptamer–substrate complex by probing the electrical features of the redox label in the rigidified 3D complex.

The thrombin aptamer undergoes a transition to a G-quadruplex structure upon binding the thrombin. Accordingly, an electrochemical thrombin aptasensor was developed by tethering a redox-active methylene blue label to the aptamer nucleic acid and then immobilizing the nucleic acid on an electrode[25] (Figure 3A). The flexible conformation of the nucleic acid chain enabled the electrical contacting of the redox label with the electrode, and the voltammetric response of the methylene blue was observed. The self-assembly of the aptamer into a G-quadruplex structure upon binding thrombin shielded the redox label from electron-transfer communication with the electrode, thus enabling the electrical detection of thrombin with a detection limit of about

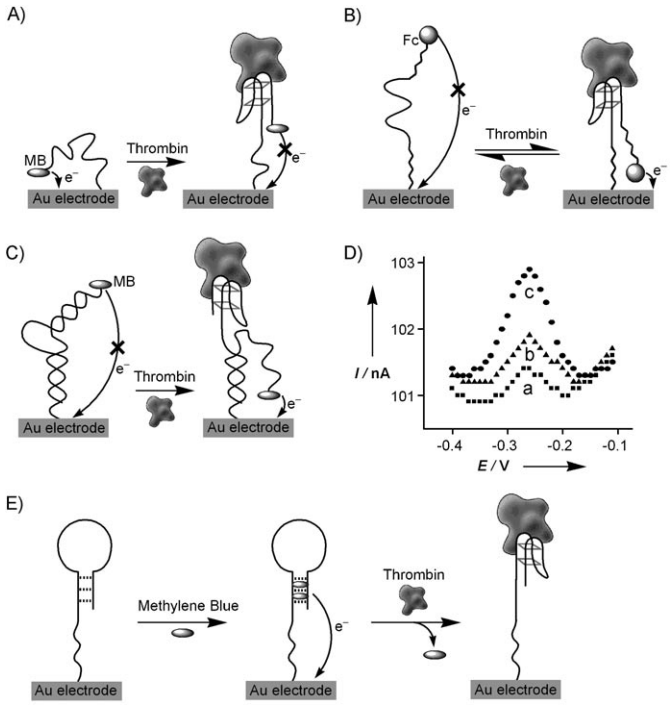

Figure 3. Electrochemical thrombin aptasensors based on the control of electron transfer between redox-labeled aptamers and the electrode. A) Shielding the electrical contact of the aptamer, which is tethered with methylene blue (MB), with the electrode upon formation of the aptamer–thrombin complex. B, C) Activation of the electrical contact of aptamers tethered with ferrocene (Fc; B) or methylene blue (C) upon formation of the respective aptamer–thrombin complex. D) Voltammograms corresponding to the analysis of thrombin by the configuration depicted in part (C): a) no thrombin, b) thrombin (10 nM), and c) thrombin (256 nM). (Reprinted with permission from reference [27]. Copyright 2005, American Chemical Society). E) Blocking the electrical response of methylene blue intercalated into the stem of a DNA hairpin as a result of the formation of the aptamer–thrombin complex.

2×10^{-8} M. The electrochemical aptasensors could be regenerated by treatment with 6 M guanidinium hydrochloride.

The detection of thrombin, by monitoring the decrease in the amperometric response of the redox label as a result of the association of thrombin is certainly a disadvantage of the sensing scheme because of a negative readout signal. To overcome these difficulties, two alternative approaches were designed. In one approach, a bifunctionalized thrombin-binding aptamer with a terminal electroactive ferrocene group as the redox label and a thiol group at the second terminus of the aptamer strand, both incorporated through hexamethylene spacers, was used to produce an electrochemical molecular hairpin. The long, flexible aptamer chain prevented electrical contact of the ferrocene label with the electrode (Figure 3B). The binding of thrombin to the aptamer domain rigidified the G-quadruplex aptamer configuration, and this resulted in the orientation of the ferrocene units towards the electrode. This led to electron-transfer communication between the electroactive ferrocene units and the electrode, and to a positive amperometric readout signal upon the detection of thrombin.[26] The sensor achieved a detection limit of approximately 5×10^{-9} M and could be regenerated by unfolding the aptamer in 1 M HCl.

A related approach[27] (Figure 3C) employed a DNA duplex assembly on an Au electrode that consisted of two double-strand domains separated by a non-complementary nucleic acid bridge. The upper duplex domain included the thrombin aptamer sequence and its complementary methylene blue functionalized nucleic acid. In the presence of thrombin, the thrombin-binding duplex was dissociated to form the G-quadruplex–thrombin complex, and the separated methylene blue functionalized nucleic acid chain revealed electrical contact with the electrode. The amperometric responses of the redox label enabled the amperometric readout of the detection of thrombin (Figure 3D). This method enabled the detection of thrombin with a detection limit of about 3×10^{-9} M. A related electrochemical aptasensor for the small molecule cocaine was also developed.[28] The thiolated cocaine-binding aptamer nucleic acid, functionalized with methylene blue as a redox label, was assembled on a Au electrode. Formation of the aptamer–cocaine complex rigidified the nucleic acid into a cocaine-binding conformation in which the redox-active units were in proximity to the electrode and yielded a voltammetric response. A further method to monitor electrochemically the aptamer–substrate interaction has used redox-active reporter units that intercalate into double-stranded DNA rather than being covalently tethered to the aptamer.[29] A nucleic acid in a hairpin configuration that includes the thrombin recognition sequence was linked to an Au electrode, and methylene blue was intercalated in the duplex stem of the probe hairpin (Figure 3E). The association of thrombin with the aptamer unit opened the hairpin structure, thus releasing the intercalated redox-active units. As a result, binding of thrombin to the interface decreased the amperometric response of the system and the process enabled the detection of thrombin with a detection limit of 11×10^{-9} M.

An indirect amplified electrochemical analysis of aptamer–protein complexes employed nanoparticles as labels for the development of electrochemical aptasensors. Metal nanoparticles were used as tracers for the analysis of nucleic acid hybridization.[30] In this approach, hybridization of a biotinylated target nucleic acid with a complementary nucleic acid associated with magnetic particles was followed by binding of the streptavidin-coated gold nanoparticles to the target DNA. Magnetic separation of the magnetic particles labeled with metal tracers, dissolution of the metal nanoparticles, and identification of the released metal ions by stripping voltammetry enabled the indirect amplified analysis of DNA. Similarly, different metal sulfide semiconductor quantum dots (QDs) were used for the parallel analysis of different DNAs.[31] Magnetic nanoparticles were modified with three different nucleic acids that acted as probes for different DNA targets (Figure 4A). The magnetic particles were then treated with a sample consisting of all three DNA chains complementary to the probe DNAs linked to the magnetic particles. Semiconductor QDs such as ZnS, CdS, and PbS, functionalized each with nucleic acids complementary to the free chains of the analyte DNAs, were then hybridized with the duplexes linked to the magnetic particles. The semiconductor QDs

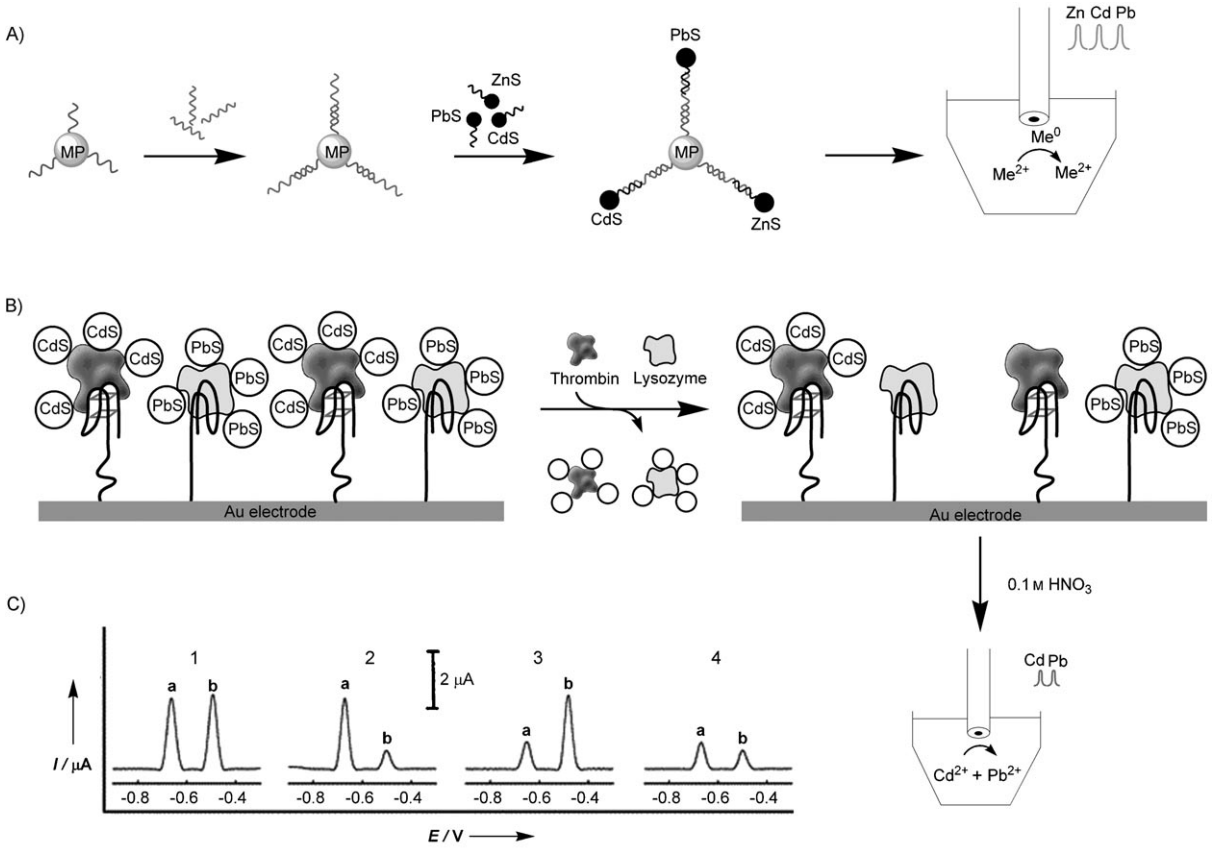

Figure 4. A) Parallel electrochemical analysis of different DNAs using magnetic particles (MPs) functionalized with probes for the different DNA targets and metal sulfides functionalized with specific nucleic acids as tracers. B) Simultaneous electrochemical analysis of the two proteins thrombin and lysozyme using a competitive assay in which thrombin modified with CdS QDs and lysozyme modified with PbS QDs are used as tracers. C) Square-wave stripping voltammograms corresponding to the simultaneous detection of thrombin (a) and lysozyme (b): 1) no lysozyme (a) or thrombin (b); 2) 1 $\mu g L^{-1}$ lysozyme (a), no thrombin (b); 3) no lysozyme (a), 0.5 $\mu g L^{-1}$ thrombin (b); 4) 1 $\mu g L^{-1}$ lysozyme (a), 0.5 $\mu g L^{-1}$ thrombin (b). (Reprinted with permission from reference [32]. Copyright 2006, American Chemical Society).

acted as tracers for the detection of the hybridization of the analyte DNA as their binding to the magnetic particles proceeded only upon the primary hybridization of the target DNA. The separation of the magnetic particles by an external magnet, followed by the dissolution of the different QDs, enabled the respective DNAs to be traced and quantitatively analyzed by the voltammetric assay of the different metal ions at their characteristic potentials. This method to encode biomolecular identity by semiconductor QDs was extended for the parallel analysis of different proteins by their specific aptamers.[32] An Au electrode was functionalized with the aptamers specific for thrombin and lysozyme (Figure 4B). Thrombin and lysozyme were modified with CdS and PbS QDs, respectively, and these were bound to the respective aptamers associated with the surface. The QD-functionalized proteins acted as tracer labels for the analysis of the proteins. In the presence of non-functionalized thrombin or lysozyme, the displacement of the respective labeled proteins preceded by the dissolution of the respective remaining metal sulfide on the surface, and the detection of the released ions by electrochemical stripping enabled then the quantitative detection of the two proteins (Figure 4C).

Interesting electrochemical aptasensors based on the interaction of ferrocene-functionalized cationic polyelectrolyte poly(3-alkoxy-4-methylthiophene) (**4**) were reported.[33] The interactions of positively charged polythiophene polyelectrolyte with double-stranded DNA and the development of optical nucleic acid sensors were extensively studied.[34] The application of the redox-labeled polyelectrolyte **4** enabled the voltammetric detection of a protein (thrombin) by the respective aptamer. In one configuration (Figure 5A), the thiolated anti-thrombin aptamer was linked to an electrode surface. The interaction of the polyelectrolyte with the thrombin aptamer yields a voltammetric response of the ferrocene unit of **4**. The association of the thrombin blocked the binding of the cationic polymer and depleted its electrochemical response. The disadvantages of the configuration are the appearance of a negative detection signal for analyzing thrombin and the unsatisfactory detection limit (higher than 1×10^{-6} M). A related improved method, albeit of increased complexity, in which **4** is applied as an electrochemical tracer is depicted in Figure 5B. According to this method, the anti-thrombin aptamer and thrombin were interacted in solution. The resulting mixture was then treated with a S1 nuclease that digested all free nucleic acid and left

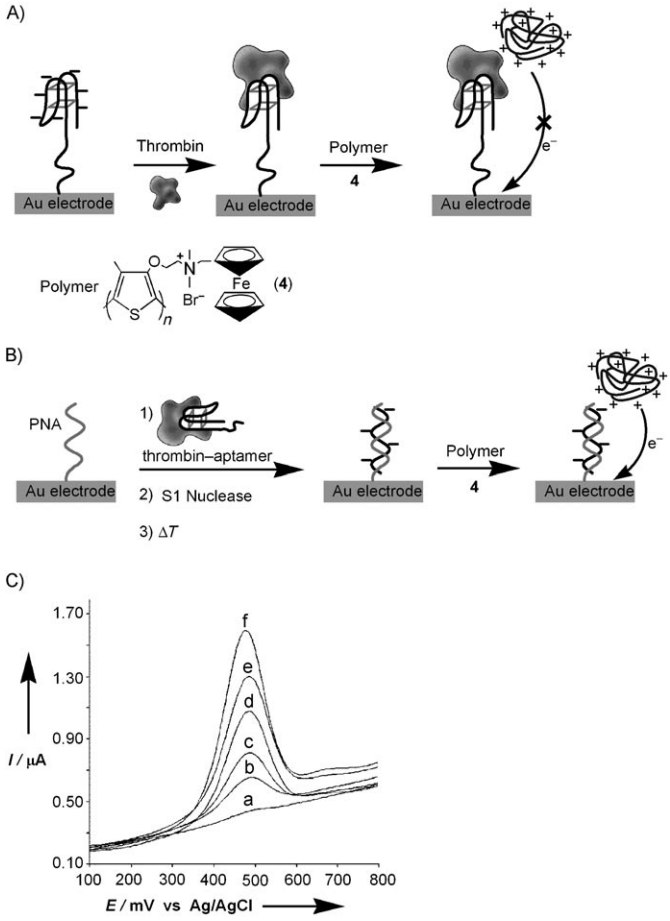

Figure 5. Electrochemical detection of thrombin by the interaction of a nucleic acid and the redox-active oligothiophene polyelectrolyte **4**: A) Blocking of the electrical contact between the polyelectrolyte and the electrode by means of the aptamer–thrombin complex. B) Separation of the aptamer–thrombin complex by the formation of a peptide nucleic acid (PNA)/open aptamer duplex on the electrode and its analysis by the redox-active polyelectrolyte. C) Voltammetric response upon the analysis of different concentrations of thrombin according to method (B): a) 0 M, b) 125 nM, c) 250 nM, d) 500 nM, e) 1 μM, and f) 2 μM. (Reprinted with permission from reference [33]. Copyright 2006, American Chemical Society).

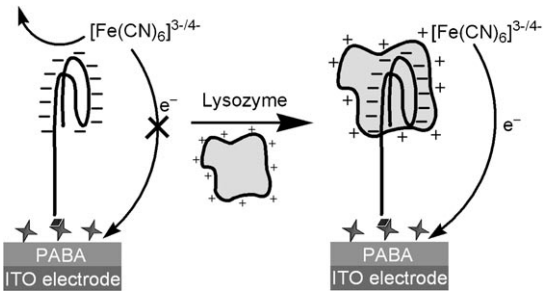

Figure 6. Impedimetric analysis of the aptamer–lysozyme complex using $[Fe(CN)_6]^{3-/4-}$ as redox label. PABA: polyaminobenzoic acid.

the aptamer–thrombin complex intact, as a result of the aptamer being protected by the protein shell. The nuclease was then inhibited by ethylenediaminetetraacetic acid, and the aptamer–thrombin complex was separated by thermal treatment. Hybridization of the dissociated aptamer with the complementary peptide nucleic acid (PNA) linked to a gold surface resulted in the negatively charged duplex structure that binds **4**. As the coverage of the duplex increased with thrombin concentration, the voltammetric responses of the electrode were enhanced as the concentration of the thrombin was elevated (Figure 5C). The method enabled the analysis of thrombin with a detection limit corresponding to 7.5×10^{-8} M.

While the previously described electrochemical aptasensors employed redox labels or catalysts as reporter units for the formation of the aptamer–substrate complexes, other label-free electrochemical aptasensors have also been developed. Faradaic impedance spectroscopy proved to be an effective method to probe biorecognition events at electrodes by monitoring changes in the electron-transfer resistances at the electrodes as a result of the biosensing events.[35] The association of proteins to electrode supports, for example, the formation of antigen–antibody complexes, were found to introduce a barrier for electrical contacting of a redox label solubilized in the electrolyte solution and the electrode, resulting in an increase in the electron-transfer resistance at the conducting support.[16,36] Similarly, the formation of double-stranded DNA on a nucleic acid functionalized electrode increases the negative charge associated with the electrode, thus repelling a negatively charged redox label solubilized in the electrolyte solution. The repulsion of the redox-active probe increases the interfacial electron-transfer resistance and enables the quantitative analysis of the hybridized DNA by monitoring the changes in the interfacial electron-transfer resistances.[37,38]

Not surprisingly, the control of the electrode surface properties by aptamer–substrate interactions has led to the development of label-free electrochemical aptasensors. The biotinylated aptamer for lysozyme was linked to a streptavidin-functionalized indium tin oxide (ITO) electrode, and the ensemble acted as a sensing interface for the lysozyme.[39] The aptamer generates a negatively charged interface that electrostatically repels an anionic redox probe such as $[Fe(CN)_6]^{3-/4-}$. The electrostatic repulsion of the redox probe from the electrode introduces a barrier for electron transfer at the electrode support and results in an increased electron-transfer resistance. The association of the protein to the "negatively charged interface" of the aptamer (at a proper pH) results in switching of the surface charge and provides a positive charge that leads to a decrease in the electron-transfer resistance (Figure 6), which enables quantitative analysis of the lysozyme. Faradaic impedance spectroscopy was also used to analyze thrombin on a thiolated aptamer functionalized Au electrode.[40] Association of the protein with the aptamer-functionalized surface insulated the electrode surface towards electron transfer with the $[Fe(CN)_6]^{3-/4-}$ redox probe solubilized in the electrolyte solution and gave rise to an increase in the electron-transfer resistance, thus enabling the quantitative analysis of thrombin.

The Faradaic impedance transduction of aptamer–protein recognition processes was also analyzed on an array configuration of electrodes.[41] The aptamer for human IgE antibody

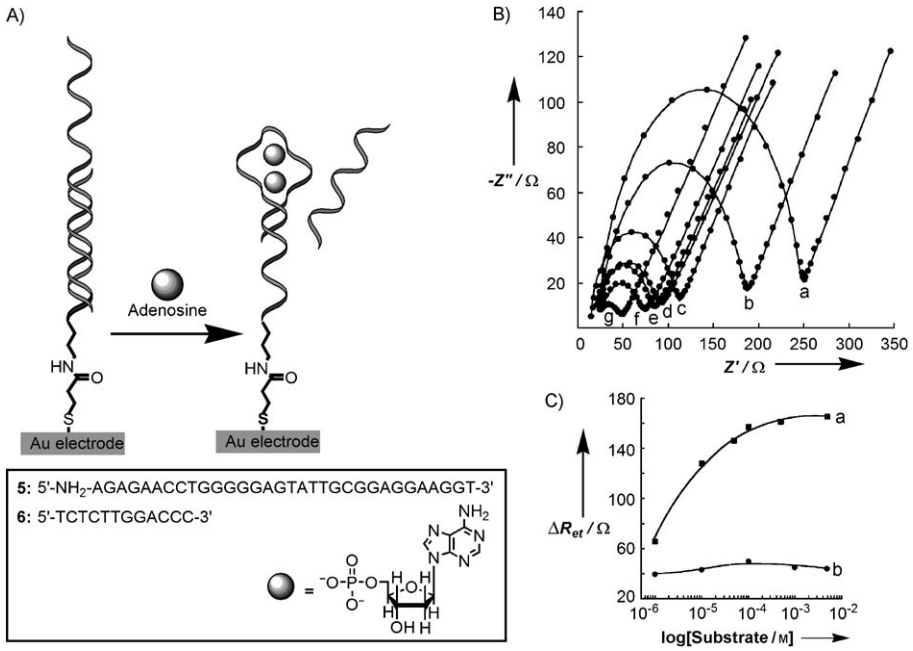

Figure 7. A) Impedimetric analysis of adenosine by the formation of the aptamer–adenosine complex through the separation of an aptamer–nucleic acid duplex associated with the electrode. The redox label in solution was $[Fe(CN)_6]^{3-/4-}$. B) Interfacial electron-transfer resistance upon the analysis of different concentrations of adenosine: a) The monolayer generated by the association of the aptamer–nucleic acid duplex to the electrode. b–f) After treatment with adenosine: b) 1×10^{-6} M, c) 1×10^{-5} M, d) 1×10^{-4} M, e) 1×10^{-3} M, and f) 1×10^{-2} M. g) The bare electrode prior to the immobilization of the aptamer–nucleic acid duplex. C) a) Calibration curve for the analysis of different concentrations of adenosine (substrate). *R*: resistance. b) Calibration curve corresponding to the control experiment in which the respective concentrations of cytidine were analyzed using the aptamer–nucleic acid duplex. (Reprinted with permission from reference [42]. Copyright 2006, American Chemical Society).

was assembled on the Au electrodes of an array, and the increase in the interfacial electron-transfer resistance upon binding of the IgE antibody using $[Fe(CN)_6]^{3-/4-}$ as redox probe was demonstrated. The interfacial electron-transfer resistance increased as the concentration of the antibody was elevated, and the IgE antibody could be detected with a detection limit of 1×10^{-10} M. Although the electrode array could be used for the multiplex analysis of different proteins, this capability has not been demonstrated yet. The electrode array was, however, applied to examine the effect of different base mutations in the aptamer sequence on the binding of the IgE antibody. By deposition of the different aptamer mutants on the electrode array and following readout of the interfacial electron-transfer resistance upon the binding of the IgE antibody, the affinity features of the different mutants to the protein were elucidated.

The use of Faradaic impedance spectroscopy as a means to identify affinity complexes between aptamers and small molecules is, however, more difficult because the reorganization of the aptamer–small-molecule complexes on the electrode yields a minute, usually undetectable, change in the interfacial electron-transfer resistance as compared to the free-aptamer-modified electrode. An approach to overcome this difficulty included the immobilization of a duplex DNA on an electrode that consisted of the amino-functionalized aptamer **5** for adenosine and a fragment complementary to the aptamer (**6**; Figure 7A). In the presence of adenosine, the duplex DNA was separated and the aptamer folded into its 3D structure that binds adenosine.[42] The separation of the duplex associated with the electrode removed the negative charge from the electrode surface thus decreasing the interfacial electron-transfer resistance in the presence of the negatively charged redox probe. The separation of the duplex DNA was controlled by the concentration of adenosine, thus enabling the quantitative analysis of the low-molecular-weight substrate (Figure 7B, C).

3. Field-Effect Transistors as Aptasensors

An alternative electronic transduction method of biorecognition events involves the application of ion-selective field-effect transistors (ISFETs). The control of the gate potential of FET devices as a result of biorecognition processes that occur on the gate surface became a common principle to develop biosensor devices.[43,44] The alteration of the charge (and as result the potential) on the gate of the ISFETs upon hybridization of the complementary nucleic acid to the gate-confined DNA was used for the label-free reagentless detection of DNA.[45,46] The general configuration of an ISFET-based biosensor includes two electrodes, source and drain, deposited on a semiconductor (e.g. Si). A gate surface is deposited on the semiconductor by its mounting on a thin insulating layer, and it separates the source and drain electrodes. The gate potential may be affected by chemical transformation occurring on it, such as charging or ionization

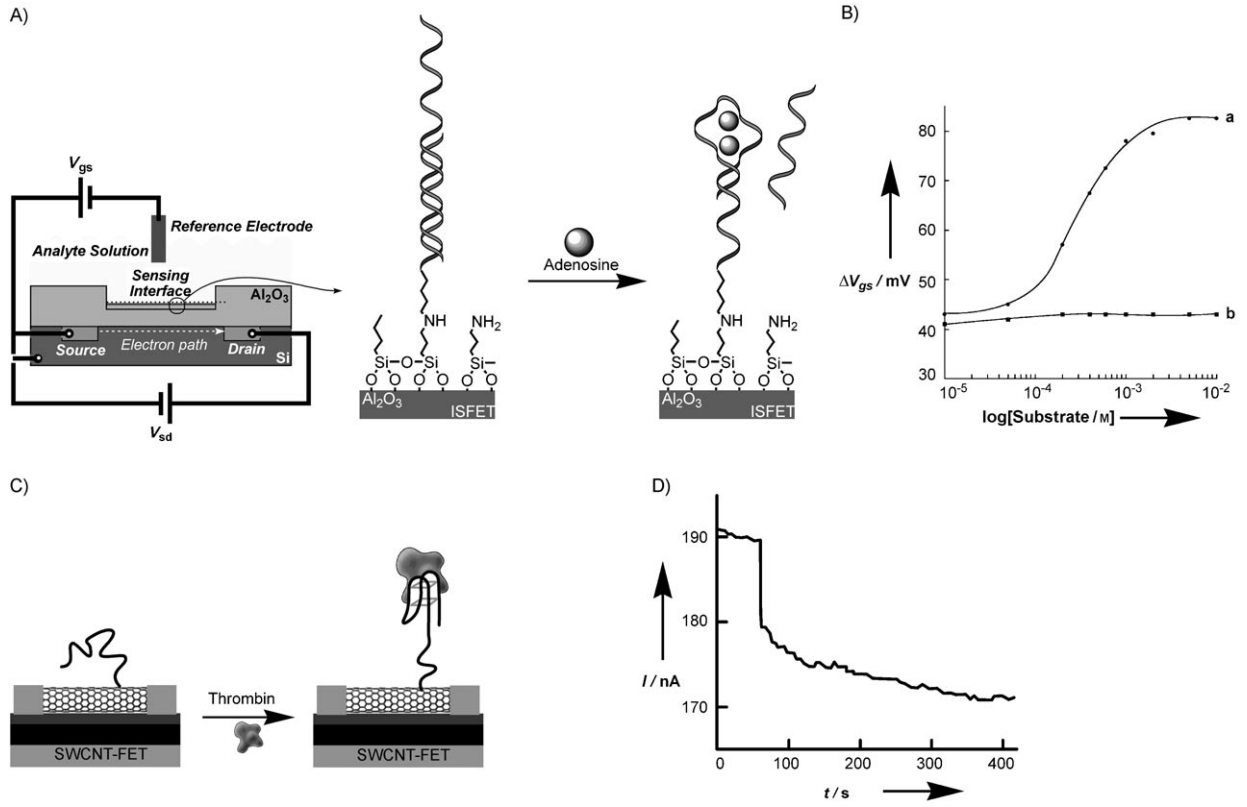

Figure 8. A) Label-free, reagentless analysis of adenosine on an ISFET device through the separation of the aptamer–nucleic acid complex by adenosine. B) Changes in the gate-to-source potentials upon the analysis of different concentration of a) adenosine and b) cytidine. (Reprinted with permission from reference [42]. Copyright 2006, American Chemical Society). C) Analysis of thrombin by the anti-thrombin aptamer-functionalized SWCNT acting as a gate of a field-effect transistor. D) Conductance changes of the aptamer-functionalized SWCNT-FET device upon analysis of thrombin. (Reprinted with permission from reference [47]. Copyright 2005, American Chemical Society).

at different pH values. For biosensing purposes, the device is immersed in an electrolyte medium and a reference electrode linked to the source electrode reflects the gate potentials. Thus, for a certain gate potential, a source-to-drain potential (V_{ds}) can be applied, and this maintains a current, I_{ds}. Upon changing the potential of the gate electrode as a result of a biosensing event, the source-to-drain current might be retained either by changing the V_{ds} potential or by altering the potential between the reference electrode and the source, V_{gs}, for compensation of the potential changes on the gate. The binding of a low-molecular-weight substrate, for example, adenosine, to its aptamer was monitored with an ISFET.[42] The Al_2O_3 gate surface was functionalized with 3-aminopropyltriethoxysilane, which was subsequently modified with glutaric dialdehyde, and then amino-functionalized nucleic acid **5** that acts as an aptamer was immobilized on the gate surface (Figure 8A). The nucleic acid **6** was hybridized with the aptamer to enhance the negative charge on the gate surface. The adenosine was analyzed by the displacement of **6** and the reorganization of the adenosine–aptamer complex on the surface. The displacement of **6** affected the local charge associated with the gate, thus enabling the transduction of the sensing of adenosine by the ISFET. Figure 8B, curve a, shows the changes in the gate-to-source potential, V_{gs}, upon analyzing different concentrations of adenosine. The detection limit for analyzing adenosine was 5×10^{-5} M. The analysis of adenosine by the aptamer revealed high specificity, and the aptamer-modified ISFET did not respond to other nucleotides, such as cytidine (Figure 8B, curve b). The method represents a label-free, reagentless analytical procedure to monitor binding reactions between aptamers and low-molecular-weight substrates.

Aptamer–protein affinity binding was also monitored on a single-walled carbon nanotube (SWCNT) FET device.[47] The monitoring of protein–protein binding on FET devices is difficult as the recognition occurs outside the electrical double-layer associated with the gate, and thus the potential changes on the gate are small. However, the dimensions of aptamers (1–2 nm) enable the perturbation of the gate potential by proteins that link to the aptamers, as the recognition binding events occur within the Debye length of the double layer ($\approx$3 nm at 10 mM ionic concentrations). Accordingly, SWCNTs were assembled between the source and drain electrodes, and the aptamer against thrombin was assembled on the carbon nanotubes (Figure 8C). The binding of thrombin to the aptamer altered the conductance through the device, thus enabling the sensing of the protein (Figure 8D). The conductance through the device was specific for thrombin, and another protein such as elastase had little effect on the conductance.

4. Microgravimetric Aptasensors Using Piezoelectric Crystals

Microgravimetric analyses on piezoelectric quartz crystals (quartz crystal microbalance; QCM) have been used as an alternative electronic transduction of aptamer–protein interactions. The frequency (f) of the quartz crystal is controlled by changes in the mass (m) associated with the crystal according to the Sauerbrey equation [Eq. (1)] (A is the piezoelectrically

$$\Delta f = -2{f_0}^2 \frac{\Delta m}{A(\mu_q \rho_q)^{1/2}} \quad (1)$$

active area, μ_q is the shear modulus, and ρ_q is the density of quartz).[48] Accordingly, the association of a protein onto aptamer-modified crystals increases the mass on the transducer, resulting in a decrease in the resonance frequency of the crystal.

An RNA aptamer specific for the HIV-1 Tat protein was immobilized on the QCM and used for the analysis of the protein with a detection limit of about 0.25 ppm.[49] Similarly, the DNA aptamer for the IgE antibody was used as a microgravimetric sensor for the analysis of the antibody.[50] The Au/quartz crystal was functionalized with a layer of streptavidin, and the biotinylated derivative of the aptamer was linked to the crystal. The IgE antibody was then sensed by the modified crystal with a detection limit corresponding to 100 $\mu g\,L^{-1}$. The performance of the microgravimetric aptasensors was compared to the classical microgravimetric immunosensors where anti-IgE was immobilized on the crystal surface. It was found that in the presence of the aptamer, the linear region for analyzing the IgE was extended tenfold as compared to that for immunosensors. This was attributed to the small size and non-protein nature of the aptamer which enabled the formation of a dense, well-oriented sensing surface for the antibody. The aptasensors for IgE could be easily recycled by washing off the analyzed protein, and it revealed specificity towards the IgE. The easy recycling of the aptasensors and the prevention of non-specific adsorption of proteins demonstrate clear advantages for alternative protein sensors that exclude antibodies as the recognition surface.

The electrochemical/QCM analysis of thrombin was also reported.[51] The biotinylated aptamer that recognizes thrombin was bound to an avidin-functionalized surface, and thrombin was bound to the aptamer (Figure 9A). The frequency changes associated with the bound thrombin or the coulometric analysis of the reduction of the methylene blue units bound to the aptamer–thrombin complex, in the presence of different bulk concentrations of thrombin, allowed the elucidation of the binding constant of thrombin to the aptamer. The electrochemical method allowed the protein to be determined with a detection limit of around 1×10^{-8} M, whereas the detection limit of the QCM method was lower at 1×10^{-9} M. The QCM analyses indicated an association constant of thrombin to the aptamer of 1.7×10^{-10} M. The use of metal-nanoparticle labels for the amplified analysis of thrombin was also reported.[52] The fact that thrombin includes two different binding sites for aptamers,[20] together with the catalytic properties of Au nanoparticles, was utilized to develop a microgravimetric QCM aptasensor for thrombin (Figure 9B). The thiolated aptamer **1** was linked to an Au/quartz crystal. The interaction of the **1**-modified Au/quartz crystal with thrombin and the subsequent association of the conjugate with aptamer-functionalized Au nanoparticles provide a primary amplification of the analysis of thrombin by the Au nanoparticles acting as a "weight label". The catalytic enlargement of the Au nanoparticles by the particle-catalyzed reduction of $AuCl_4^-$ by 1,4-dihydronicotinamide adenine dinucleotide (NADH)[53] provided a further (secondary) amplification of the thrombin sensing. Upon analyzing thrombin at a concentration of 2×10^{-9} M, the primary amplification step resulted in a frequency change of -30 Hz, whereas the secondary amplification step altered the crystal frequency by -900 Hz (9 MHz crystals were employed).

An alternative approach to monitor aptamer–protein complexes includes the application of surface acoustic wave (SAW) Love-wave sensors.[54] The loading of the surface of the sensors with proteins alters the velocity of the propagating acoustic wave, thus resulting in the reduction of the resonance frequency of the crystal or the alteration of the phase shift between the output and input signals. The Love-wave SAW sensors apply horizontal acoustic shear waves that minimize

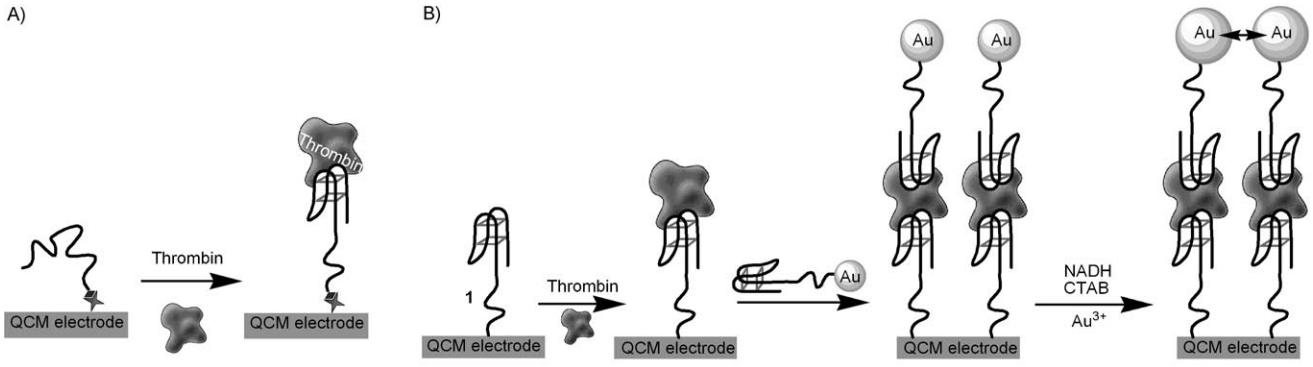

Figure 9. Migrogravimetric analysis of thrombin on piezoelectric crystals: A) Analysis of the aptamer–thrombin complex. B) Amplified analysis of thrombin by association with an aptamer functionalized with Au NP labels and subsequent catalytic enlargement of the NPs. CTAB: cetyl triethylammonium bromide.

the energy dissipation with the solvent environment, and the method has proved to be effective to probe submonolayer coverage.[55] Accordingly, the method was successfully applied to analyze thrombin or the HIV-1 Rev peptide by the respective aptamers. The DNA aptamer for thrombin or the RNA aptamer for HIV-1 Rev peptide were immobilized on the SAW crystals, and by monitoring the phase shift between the input and output signals the respective proteins could be analyzed with a detection limit of 75 pg cm^{-2}.

5. Conclusions and Perspectives

Electronic aptasensors reveal certain advantages when compared to optical aptasensors. For example, the possibility to couple amplifying catalytic or biocatalytic labels to the aptamer–target complex enables the amplified detection of the target substrate, and thus enhances the sensitivity of the sensing processes. Recent advances have also demonstrated the electronic label-free detection of the target substrate by aptamers, and thus fluorescent labels may be excluded.

The possible variations in the sequences of oligonucleotides enable an enormous diversity of aptamers for almost any molecule or macromolecule; indeed, DNA or RNA aptamers for hundreds of targets have been developed.[56] The impressive selectivity of aptamers suggests that evolutionary selected binding of oligonucleotides might conquer the selectivity of antibodies. For example, an anti-theophyllin aptamer revealed an approximate 10^4-fold enhancement in binding affinity towards theophyllin as compared to caffeine, which differs only by a single methyl group in the molecular structure.[2] Even though substantial progress was accomplished in the application of aptamers in analytical chemistry, several exciting opportunities still exist in the field of aptasensors: The use of synthetically modified nucleotides as co-components in the selected evolution of the aptamers will lead to nuclease-resistant oligonucleotides and eventually to aptamers of higher affinities to the target substrates. Furthermore, we have emphasized the use of SELEX for the synthesis of aptamers. Nonetheless, similar to the scientific efforts to elicit catalytic antibodies, the SELEX procedure has been applied to synthesize catalytic DNA/RNA (DNAzymes/RNAzymes) by selecting oligonucleotides to the respective reaction.[57] The integration of the binding properties of aptamers with DNAzyme units might yield hybrid systems that include a built-in amplifying label. Such molecular hybrids would reveal superior sensing functions by combining evolutionary selected binding and catalytic properties of nucleic acids.

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