

# New trends in aptamer-based electrochemical biosensors

## Review Article

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**Abbreviations:** Platelet-derived growth factor BB (PDGF-BB); reverse-transcription PCR (RT PCR); self-assembled monolayers (SAMs); Systematic Evolution of Ligands by EXponential enrichment, (SELEX)

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## Summary

The analytical characteristics of aptamers are comparable with those of antibodies for the development of biosensor technology. However, aptamers offer some crucial advantages over antibodies such as selection capability for a variety of targets, easy synthesis, improved reproducibility and stability, simple modification for immobilization to solid supports and enhanced selectivity. This article reviews aptamer technology as well as aptamer-based assay configurations and goes on to explore reported applications in electrochemical aptasensors.

## I. Introduction

Biosensor technology holds a great promise for the healthcare market, the security sector, the food industry, environmental and veterinary diagnostic; harnessing the specificity and sensitivity of biological-based assays packaged into portable and low cost devices which allow for rapid analysis of complex samples in out-of-laboratory environments. However the application of biosensors lags far behind the fundamental research; the challenges facing this basic technology are associated with sensitive detection of specific molecules in samples, stability issues, quality assurance, instrumentation design and cost considerations (Velasco-Garcia and Tottram, 2003).

The main biological sensing materials used in biosensor development are the couples enzyme/substrate and antibody/antigen. These are limited by temperature, sensitivity, stability, batch-to-batch variation, large size and difficulty in production. Recent advances and developments in the aptamer area offer a powerful alternative approach involving the use of small RNA or DNA molecules that bind to specific targets with very high affinity and specificity. Aptamer receptors are a novel entity of undeniable potential in analytical applications and can complement or substitute antibodies or offer

applications where the later are not compatible (Tombelli et al, 2005, 2007).

Despite the fact that development of aptasensors has been boosted by using optical and acoustic transducers, this review summarizes the recent developments in the design of electrochemical aptamer-based affinity sensors. In comparison with other detection systems, the electrochemical detection combines a high sensitivity, direct electronic signal production, fast response, robustness, low cost, the possibility of miniaturization and simultaneous multianalyte detection.

## II. Aptamers

As aptamers approach 20 years since they were originally described (Ellington and Szostak, 1990; Tuerk and Gold, 1990), they are currently receiving a wider recognition in the literature as research reagents, inhibitors, imaging or diagnostic agents (Luzi et al, 2003; Hamula et al, 2006). Aptamers are short, single stranded oligonucleotides, which inherently adopt stable three dimensional sequence-dependent structures. This intrinsic property makes them efficient binding molecules, capable of binding to an array of molecular targets ranging from small ions and organic molecules to large glycoproteins

and mucins (Ferreira et al, 2006). Aptamers are a novel and particularly interesting targeting modality, with the ability to bind to a variety of targets including proteins, peptides, enzymes, antibodies and cell surface receptors, as well as small molecules ranging from glucose and caffeine, to steroids to TNT. Aptamers are single stranded oligonucleotides that vary in size between 25-90 bases long and adopt complex secondary and tertiary structures, which facilitate specific interactions with other molecules. They are derived from vast combinatorial libraries through selective targeting and competitive binding. There are two different configurations of aptamers: (i) linear and (ii) molecular beacon. Aptamers with a linear configuration maintain in certain physicochemical conditions a typical 3-D conformation with specific binding sites for the target molecule. On the other hand aptamers with a molecular beacon configuration initially form a loop that changes conformation following binding to the analyte of interest.

Aptamers offer unique benefits compared to other targeting agents; not only they bind specific ligands with high affinity and selectivity, but aptamers can be easily selected using in vitro techniques and are chemically synthesized, overcoming the use of animal for their production. In comparison to antibodies, aptamers are purified to a very high degree of purity, which eliminates the batch-to-batch variation found in antibodies. Aptamers have higher temperature stability (stable at room temperature) and because of their small size, denser receptor layers could be generated. The animal-free production of aptamers is especially advantageous in cases where the immune response can fail when the target molecule (e.g. a protein) has a structure similar to endogenous proteins or when the antigen consists of toxic or non-immunogenic compounds. Aptamers are relatively

stable under a wide range of buffer conditions and resistant to chemical degradation, although, due to their DNA or RNA constitution, they are sensitive to hydrolytic digestion by nucleases. Aptamers have been modified into nuclease-resistant moieties by modification of the ribose ring at the 2'-position or by the specific modification of the pyrimidine nucleotide (Pieken et al, 1991; Heidenreich and Eckstein, 1992; Kusser, 2000). It is also possible to chemically modify aptamers to facilitate covalent conjugation to reporters and nanoparticles with 5' or 3' amino, biotin or thiol groups. These characteristics make them extremely attractive as alternatives to antibodies and peptides for use in assays, or as diagnostic agents.

### A. The SELEX process

Aptamers are typically isolated from combinatorial libraries by a process of in vitro evolution, termed SELEX (Systematic Evolution of Ligands by EXponential enrichment). This procedure is an in vitro evolutionary selection process that allows the isolation of aptamer(s), with unique binding properties, from a large library of oligonucleotides through iterative cycles of (i) interaction of a large library of aptamers with the target molecule, (ii) separation of bound from unbound aptamer species, (iii) elution of bound aptamers and (iv) PCR amplification of the binding aptamers for further selection rounds (Figure 1 for an example of the process).

An aptamer library usually consists of a variable region (20-40 nucleotides) flanked by known primer sequences on either end for the amplification during the SELEX procedure. The variable region makes up to  $10^{15}$  different sequences which, combined with the innate ability of oligonucleotides to form stable sequence-

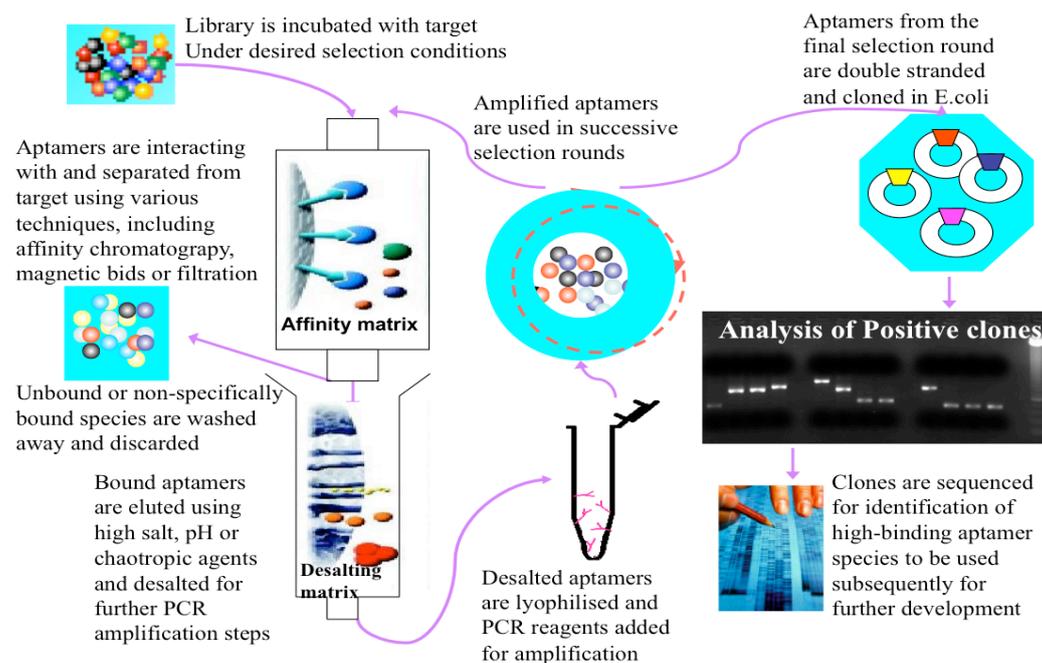


Figure 1. The SELEX process

dependent structures, provide an array of molecular shapes available for the selection process (Khan and Missailidis, 2008). In the selection steps, the library is incubated with the immobilised target. Unbound or weak-binding species are removed and bound aptamers are eluted using high salt, temperature, chaotropic agents or other such conditions that would affect molecular structure or disrupt molecular interactions. Eluted aptamers are subsequently amplified by PCR (DNA) or reverse-transcription PCR (RT PCR) using primers complementary to the flanking sequences in the aptamer library. The enriched pool of binding species forms the pool for the next round of selection. Repeated selection and amplification steps allow identification of the highest binding species, through competitive binding. The selection and amplification step constitutes one round or cycle in a typical SELEX procedure, with anything between 1 and 15 cycles often described in the literature. Counter- or negative selection steps can ensure that the finally selected aptamers are very specific for their target and do not interact with homologous proteins or chemically closely-related molecular targets (Missailidis, 2008).

Selected aptamers are subsequently cloned and sequenced to identify the sequence of the binding species and their interactions are usually characterised by a variety of analytical methodologies, prior to move into the various applications they were originally destined for. Selected aptamer can be easily produced by solid phase synthesis and appropriate modifications can be introduced at this stage to confer additional properties to the selected aptamers, such as nuclease resistance (Figure 2), cross-linking ability or improved pharmacokinetic properties.

Although SELEX has been the initial methodology associated with aptamer selection and has remained a robust and powerful technique, which has been adapted to various systems and targets, a number of other methodologies have also emerged for the selection of aptamers. Such “non-SELEX” based methods for the selection of aptamers include capillary electrophoresis methodologies (Berezovski et al, 2005; Drabovich et al, 2005), isolation of aptamers with predefined kinetic and thermodynamic properties of their interaction with the target, without the need for amplification, allowing the use of libraries which are difficult or cannot be amplified, or computational methods, which are particularly important in selecting aptamers with inhibitory activities or sequences that undergo ligand dependent conformational changes (Ikebukuro et al, 2005).

The SELEX procedure and subsequent technologies for aptamer selection have offered the tools for the designing of aptamers that have found a range of diagnostic applications (Khan and Missailidis, 2008). Such applications include Photo-SELEX (www.somalogic.com) and SELEX NADIR (Winters-Hilt, 2006) using optical probe reporting or nanopore reporting mechanisms respectively, aptamer microarrays (Cho et al., 2005), currently in the market by LC Sciences (www.lcsciences.com), fluorescent aptamers in chips and microspheres (Kirby et al, 2004; Potyrailo et al., 1998), fluorescent sensors for small molecule recognition (Ozaki et al, 2006; Yamana et al, 2003), quantum dots (Liu et al,

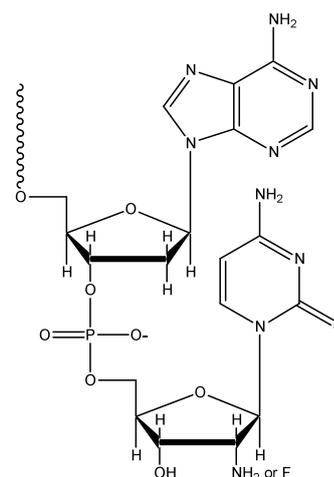
2007; Levy et al, 2005; Choi et al, 2006; Ivanovic et al, 2007), colorimetric detection (Liu and Lu, 2004; Cho et al, 2006; Liu and Lu, 2006), electrochemical detection (Lai et al, 2007; Xiao et al, 2005; Papamichael et al, 2007; Mir et al, 2006) and piezoelectric quartz crystal sensors (Bini et al, 2007).

The above methods, fluorescent, electrochemical and colorimetric detection, have also been used in molecular switch type sensors or modular sensor assemblies, where the aptamers usually change conformation upon binding to either emit a fluorescent signal based on an aptamer beacon on sensor, or through non-covalent interaction with the fluorescent label, triggering an electrochemical sensor or leading to change of colour (Stojanovic and Kolpashchikov, 2004; Stojanovic et al, 2001; Baker et al, 2006; Zuo et al, 2007; Stojanovic and Landry, 2002; Frauendorf and Jaschke, 2001), with particular sensitivities in the recognition of small analytes.

Aptamers have also been used in enzymatic sensing, without the use of any label or signal related directly to the aptamer. These applications remain based on changes in the conformation of bifunctional aptamers that recognise the target ligand and an enzyme or ribosome. The binding of the aptamer to the ligand results in conformational changes that affect enzymatic activity or protein expression, and it is the later that is subsequently measured (Ogawa and Maeda, 2007; Yoshida et al, 2006; Yoshida et al, 2006) or utilises an enzyme to ligate proximally bound aptamers to large protein targets and allow their subsequent PCR amplification (Fredriksson et al, 2002).

### III. Aptamer immobilisation

Aptamers can certainly be used as molecular recognition elements in affinity sensing. The small size of aptamers provides advantages over antibodies: (i) a greater



**Figure 2.** An amino or fluoro modification at the 2' position of the sugar can confer the oligonucleotide aptamer stability against nuclease degradation. An alternative to using modifications at the 2' of the sugar (whether at the 3' or 5' end of the aptamer, or both) for nuclease resistance is to use a flipped base added to the end of the aptamer.

surface density of receptors and (ii) multiple binding to target molecules for sandwich assays.

The method of immobilization of aptamers to a solid support affects the sensitivity of the aptamer to the target molecule. Thus, the selected method should maintain the binding affinity and selectivity that the aptamers display in solution (Balamurugan et al, 2008).

Aptamers can be attached to the solid support at either the 5'-end or the 3' end. Both positions have been reported as being used for aptasensor development. However, there are very few studies looking at the effect of the two types of end attachment. Recent work suggests that it depends on the particular aptamer (Cho et al, 2006), although for biological targeting it may be that the 3' end is more suitable, since the 3' end is the primary target for exonucleases, and thus its coupling to the solid support would simultaneously confer resistance to nucleases.

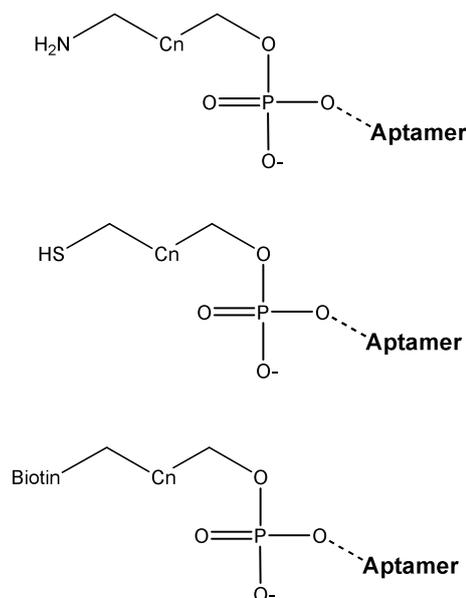
Gold is used for many electrochemical measurements. Direct attachment of aptamers to gold surfaces could be achieved by using a thiol-alkane linked to the aptamer sequence. The gold surface could also be functionalized and the type of chemistry selected is dependent on what type of terminal functional group is linked to the aptamer (amine, thiol or biotin termini; Figure 3).

Gold surfaces functionalized with self-assembled monolayers (SAMs) can address the nonspecific adsorption of aptamer to the surface, which is a particular problem for long oligonucleotides with larger numbers of amine groups. Avidin-biotin technology has also been exploited for aptamer immobilization. Streptavidin can be physically adsorbed or covalently immobilized onto the support and the method mainly requires incubation of the biotin-tethered aptamer with the modified substrate. Studies of the anti-thrombin aptamer revealed this biocoating method gives best results regarding sensitivity compared to other immobilization strategies (Hianik et al, 2007).

#### IV. Electrochemical assays

In principle, aptamers can be selected for any given target, ranging from small molecules to large proteins and even cells. When aptamers bind small molecular targets, these get incorporated into the nucleic acid structure, buried within the binding pockets of aptamer structures. On the other hand, large molecules (e.g. proteins) are structurally more complicated, allowing aptamer interactions at various sites via hydrogen bonding, electrostatic interactions and shape complementarity. The use of aptamers as bio-recognition elements for small molecules has not been reported as extensively as for protein targets.

Mainly two different assay configurations have been reported to transduce these target-binding aptamer events: (i) single-site binding and (ii) dual-site binding (Song et al, 2008). Small molecules are often assayed using the single-site binding configuration. Protein targets can be assayed via both single-site and dual-site binding. The dual-site binding assay is commonly known as the sandwich assay. Normally, the target molecule is sandwiched between a pair of aptamers that bind to different regions of the large



**Figure 3.** Standard nucleic acid modifications used for aptamer immobilisation. Most of the common modifications are linked via the phosphate group of the oligonucleotide aptamer. Various lengths carbon chains are used that can offer higher or lower flexibility.

molecule. One aptamer is immobilized on a suitable solid support to capture the target while the other aptamer for detection is conjugated to a catalytic label. Enzymes, inorganic or organic catalysts or nanoparticles are often used for electrochemical detection. In some cases, when there is only one aptamer for the molecule of interest, antibodies have been reported to be used instead of the second aptamer (Ferreira et al, 2008). If the target protein contains two identical binding sites, the selection of a single aptamer still allows the development of a sandwich assay.

Displacement assays have been also proposed to overcome the more challenging detection of small molecules. Affinity interactions between aptamers and small ligands are weaker than interaction with large molecules (with dissociation constants in the  $\mu\text{M}$  range, in comparison with constants for large molecules that are in the  $\text{pM}$ - $\text{nM}$  range). The presence of the small target could induce the separation of two strands of a duplex nucleic acid (one strand being the aptamer immobilised to a solid support). Another strategy could rely on the displacement of the aptamer from its complex with the immobilised target molecule when the molecule is present in solution (De-los-Santos-Alvarez et al, 2008).

Induced-fit conformational changes of the aptamer after binding to the target molecule can also be used to monitor a bio-recognition event by tagging the aptamer (Figure 4). The use of labels requires precise knowledge of the aptamer folding mechanism after binding to the target and the binding sites. In the case of a redox active marker, the accessibility of the label to the conducting support is associated with the tertiary structure of the aptamer before and after the binding event. However, for small molecules, this strategy is not always viable,

because the aptamer 3D structure could only be slightly perturbed after the ligand interaction.

Redox-active reporting labels could not be covalently tethered to aptamers. Methylene blue has been intercalated into the double-stranded DNA domain of a hairpin configuration aptamer. The binding of the target with the aptamer opens the hairpin structure and releases the intercalated methylene blue. As a result, the amperometric response decreased with the addition of the analyte. This approach is known as “label-free” method (**Figure 5**). Related approaches use cationic redox-active reporting units bound to the electrode via electrostatic interactions with the DNA aptamer phosphate backbone. The binding of the target molecule with the aptamer blocked the binding of the cationic reporting units and the electrochemical response decreased. The main disadvantage of these latter approaches is a negative detection signal.

Recently, nanomaterials are also providing novel electrochemical sensing approaches. Single-walled carbon nanotube field-effect transistor sensors were developed to monitor aptamer-protein binding studies. Aptamers are well suited for FET sensing due to their small size (1-2 nm) and recognition occurs inside the electrical double-layer associated with the gate (within the Debye length). The single-walled carbon nanotubes were assembled between source and drain electrodes and the aptamers were immobilized to these nanomaterials. In this label-free approach, the binding of the target molecule to the aptamers altered conductance through the device. The ease

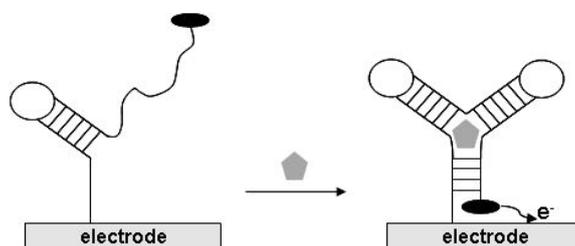
of miniaturization of these sensing devices opens up the feasibility of high-throughput assays in microarrays.

Nanoparticles have also been reported as catalytic labels, instead of enzymes, and carriers for ultrasensitive electrochemical detection; because one nanoparticle contains a large number of aptamers, the target binding process is amplified.

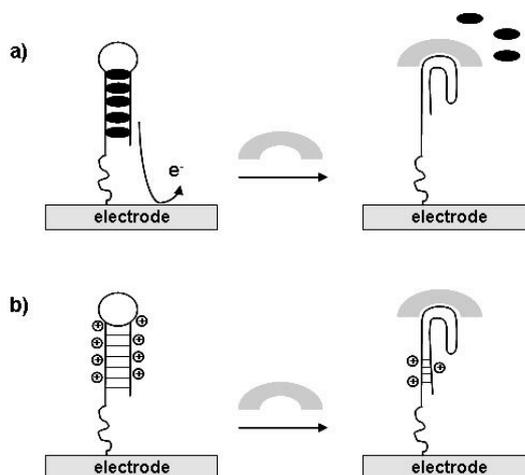
Impedance spectroscopy has been the most frequently used electrochemical method in the development of electrochemical aptasensors and has shown excellent sensitivity, achieving limit of detection of fM. However, despite the fact that the analytical technique is simple to perform, the data fitting remains a bit complicated. Easier data processing and faster response could be achieved with chronoamperometry, but the limit of detection will be higher and in the nM range.

#### IV. Applications of electrochemical aptasensors

Aptamer publications have now appeared in the literature using most of the electrochemical transducers. The majority of aptamer work on electrochemical sensors is focused on amperometric transducers, but there have been references on aptamers used in impedimetric, FET and recently potentiometric sensors. Furthermore, a lot of the work on the aptamers in electrochemical sensors has been on the model protein, thrombin, which is one of the best characterised complexes in the aptamer literature.



**Figure 4.** Assays based on induced-fit conformational changes of aptamers.



**Figure 5.** Label-free electrochemical assays based on: (A) methylene blue intercalated into the DNA aptamer and (B) cationic redox-active reporting units bound to DNA aptamer phosphate backbone.

These have provided proof of principle concepts as to how aptamers could be developed in novel sensors. However, a number of other systems have also now been described, which will be presented in this review.

### A. Electrochemical aptasensors for the model protein

The thrombin-binding aptamer (15-mer, 5'-GGTTGGTGTGGTTGG-3') was the first one selected in 1992 by Block and colleagues and its structure has been well characterized and studied. The folded structure in solution is composed of two guanine quartets connected by two T-T loops spanning the narrow grooves at one end and a T-G-T loop spanning a wide groove at the other end (known as the G-quartet structure). This anti-thrombin aptamer has been extensively used as the model oligonucleotide by many researchers to demonstrate the wide applicability of aptamers as bio-recognition elements in biosensors.

In the literature, many different electrochemical aptasensors for thrombin detection have been reported. The most straightforward configuration is based on the immobilization of a thiol terminated aptamer on a gold electrode. The aptamer-thrombin interaction is transduced by the electrochemical quantification of p-nitroaniline produced by the thrombin's enzymatic reaction. Thrombin has two electropositive exosites both capable of binding the aptamer, allowing the development of an electrochemical sensor system in a sandwich manner. The thiolated aptamer was immobilized on a gold electrode and, after incubation with the thrombin, a second incubation step with an HRP labelled aptamer took place. Electrochemical detection of HRP was performed using  $H_2O_2$  and a diffusional osmium based mediator. A similar aptasensor system in sandwich manner for thrombin was developed based on the aptamer for detection, labelled with pyroquinoline quinine glucose dehydrogenase, and the electric current generated from glucose addition after the formation of the complex on a gold electrode (Ikebukuro et al, 2005). Another strategy for the thrombin sensing is the direct immobilization of the protein on the electrode surface. After the incubation with biotin-labelled aptamer and then with streptavidin-HRP, the electrochemical detection is performed using  $H_2O_2$  and a diffusional osmium-based mediator. The latter approach achieved the lower limit of detection, 3.5 nM (Mir et al, 2006).

Mir and colleagues also developed in 2008 a chronoamperometric beacon biosensor based on a ferrocene-labelled thiol-aptamer. The aptamer adopts a 3-D conformational change after binding the thrombin, allowing the ferrocene label to approach to the gold electrode. The interaction is detected via a micropoxidase mediated electron transfer between the label and the electrode surface. The system was demonstrated with impedance spectroscopy and chronoamperometry measurements, achieving a limit of detection of 30 fM with the impedance spectroscopy (Mir et al, 2008).

Methylene blue has also been used as an electrochemical marker. The beacon aptamer surface was

prepared following formation of 11-mercaptoundecanoic acid self-assembled monolayer on gold electrode. Methylene blue was intercalated on the aptamer by the interaction with two guanine bases. Binding of the thrombin is correlated with the decrease in electrical current intensity in voltammetry. The estimated detection limit of the target thrombin was 11 nM (Bang et al, 2005).

The modification of antibodies is difficult, costly and time consuming; however researchers have been using conventional polyclonal antibodies as a capturing probe and labelled-aptamers as the detection probe in new sandwich approaches for protein detection. Kang and colleagues reported in 2008 a modified electrochemical sandwich model for thrombin, based on capturing antibody immobilized onto glassy carbon electrodes with nanogold-chitosan composite film and Methylene blue labelled aptamer as the electrochemical detection probe.

Lu and colleagues described in 2008 an electrochemical aptasensor for thrombin that is not based on the target binding-induced conformational change of aptamers. The thrombin-binding aptamer is first assembled onto a gold electrode and then hybridized with a ferrocene labelled short aptamer-complementary DNA oligonucleotide. The binding of the thrombin to the aptamer destroys the double-stranded DNA oligonucleotide and leads to the dissociation of the label short complementary DNA oligonucleotide from the electrode surface, resulting in a decrease in the differential pulse voltammetry responses at the electrode (Lu et al, 2008). This strategy is based on the stronger binding affinity of the aptamers towards their targets rather than to the short aptamer-complementary DNA oligonucleotide labelled with electroactive moieties.

The majority of the work performed on aptamer-based electrochemical biosensors is based on aptamers labelled using redox compounds, such as methylene blue, and catalysts such as horseradish peroxidase. However, nanoparticle-based materials offer excellent prospects for a new signal amplification strategy for ultrasensitive electrochemical aptasensing. Platinum nanoparticles have been reported as catalytic labels when linked to a thiolated aptamer. The nanoparticles catalysed the electrochemical reduction of  $H_2O_2$  and the resulting current enabled the amplified detection of thrombin sandwiched between the aptamer on the electrode surface and the aptamer labelled with the nanoparticles (Polsky et al, 2006). Gold nanoparticles offer several advantages such as electrical conductivity, biocompatibility, ease of self-assembly through a thiol group, increase electrode surface area and amount of immobilized capturing probe. Gold nanoparticles have been used as an electrochemical sensing platform for direct detection of thrombin. The aptamer was immobilised on a screen-printed electrode modified with gold-nanoparticles by avidin-biotin technology. The gold-nanoparticles surface status is evaluated by the Au/Au oxide film formation with cyclic and stripping voltammetry. Gold nanoparticles signal changed with the deposition of bilayers due to differences in electron transfer efficacy and availability of buffer oxygen. Aptamers prefer to adopt the G-quarter structure when binding with thrombin and the

conformational changes made double strand DNA zones appear and facilitated the electron transfer from solution to the electrode surface, based on the double stranded DNA's ability to transport charge along the nucleotide stacking (Suprun et al, 2008). The detection limit of this novel approach is in the nM range. However, the aptasensor measured directly binding events and opened 4 orders of magnitude the operating range of protein concentration.

Assays coupling aptamers with magnetic beads for the aptamer or target immobilisation before the electrochemical transduction have also been proposed (Centi et al, 2008). The use of magnetic beads improved the assay kinetics due to the beads being in suspension and also minimized matrix effect because of better washing and separation steps.

An ultrasensitive electrochemical aptasensor for thrombin in a sandwich format of magnetic nanoparticle-immobilized aptamer, thrombin and gold nanoparticle-labelled aptamer was reported by Zheng and colleagues in 2007. The magnetic nanoparticle-immobilized aptamer was used for capturing and separating the target protein. The gold nanoparticle-labelled aptamer offered the electrochemical signal transduction. The signal was amplified by forming a network like thiocyanuric acid/gold nanoparticles to cap more nanoparticles per assay, lowering the detection limit to the aM range

## B. Other targets

Aptamer have been selected against a wide range of targets with typical binding affinities in the nanomolar to picomolar range. Recently, electrochemical aptasensors have been reported to detect proteins, hormones and drugs.

Papamichael and colleagues described in 2007 a disposable electrochemical aptasensor for Immunoglobulin E, a key marker of atopic diseases (such as asthma, dermatitis and pollenosis). The sensor incorporates a competitive format for IgE detection using a biotinylated form of the aptamer. A standard, indirect method was used where competition between surface-bound IgE and IgE in solution proceeded for the aptamer. The electrochemical detection is achieved by the use of an extravidin-alkaline phosphatase label. After careful optimization of conditions (buffer pH, ionic strength, additional ions and proteins), the aptasensor was performing at levels suitable for human testing ( $>300\text{ng ml}^{-1}$ ).

Platelet-derived growth factor BB (PDGF-BB) is one important cytokine involved in neural inflammation and was selected as target for the development of an electrochemical aptasensor based on capacitance change induced by aptamer-protein specific binding, measured by non-faradic impedance spectroscopy. The biosensor detection limit was 40 nM. Electrochemical impedance spectroscopy is a very attractive method for in vivo diagnostics, due to its high sensitivity and label free characteristics (Liao and Cui, 2007). A similar electrochemical detection was also reported to a tuberculosis-related cytokine, the interferon- $\gamma$ . The aptamer-based electrochemical impedance biosensor successfully detected interferon- $\gamma$  to a level of 100 fM

with an RNA aptamer and 1 pM with a DNA aptamer probe (Min et al, 2008).

Electrochemical aptasensors for 17- $\beta$  estradiol have also been reported. The selected biotinylated DNA aptamer was immobilized on a streptavidin-modified gold electrode. The chemical binding of the hormone to the aptamer was monitored by cyclic and square wave voltammetry. When the 17- $\beta$  estradiol interacted with the aptamer, the current decreased due to the interference of the bound target molecule with the electron flow produced by a redox reaction between ferrocyanide (the mediator) and ferricyanide. The linear range of this aptasensing device was 1-0.01 nM of 17- $\beta$  estradiol (Kim et al, 2007).

Cocaine has been detected by an electrochemical aptasensor incorporating gold nanoparticles onto the surface of a gold electrode. The thiol-derivative aptamer was self-assembled onto the gold nanoparticles. The aptamer was also functionalized at the other termini of the strand with a redox-active ferrocene moiety. The cocaine binding to the aptamer induces the conformational change of the aptamer, bringing the redox tag in close proximity to the electrode, leading to an increase in the current (Li et al, 2008). Methylene blue tagged aptamer has been also explored for the detection of cocaine (Baker et al, 2006).

A novel adenosine aptasensor was reported based on the structure change of an aptamer probe immobilized on a gold electrode. After the binding aptamer-target nucleoside, a higher surface charge density and an increasing steric hindrance were obtained that reduce the diffusion of  $[\text{Fe}(\text{CN})_6]^{3-}/[\text{Fe}(\text{CN})_6]^{4-}$  towards the electrode surface, resulting in a decrease of the current. The biosensing surface was easily regenerated and the aptasensor limit of detection was 10 nM (Zheng et al, 2008).

## C. Aptasensor arrays

Some of the aptamer-based biosensor technology described in this review could be transferred from single-analyte devices to electrochemical methods offering the possibility of simultaneous measurements of a panel of targets. Wang reviewed the use of metal nanoparticles as tracers for the analysis of nucleic acid hybridization. Magnetic nanoparticles were linked to different probe DNAs and incubated with samples containing different DNA targets. Semiconductor quantum dots were functionalized each with different nucleic acids complementary to the free chain of the target DNA. After dissolution of the metal nanoparticles, the identification of the metal ions by stripping voltammetry enabled the analysis of the different DNA targets (Wang, 2003).

Thrombin and lysozyme were detected in parallel using a competitive assay in which thrombin and lysozyme were modified with different semiconductor quantum dots (Hansen et al, 2006). Specific aptamers were immobilized on a gold electrode and bound to the respective labelled protein. In the presence of unlabelled protein in the sample, the quantum-dot functionalized protein is displaced from the electrode into solution. The dissolution of the remaining metal ions on the surface and the electrochemical detection of the released ions enabled the quantitative detection of the proteins.

## IV. Conclusions

Aptamers have been widely used in a variety of diagnostic and sensor applications, offering a variety of possibilities for aptamer-based sensors in early disease diagnosis and prognosis, substance control, environmental measurements or national security applications on measurements of explosives or potential infectious agents. Yet, despite the advances and the huge body of literature documenting the success of the technology, the commercial application of aptamers in the field of diagnostics remains relatively undeveloped, not least due to the exclusive IP portfolio, and the fact that there is a vast antibody-based diagnostic market and a certain degree of hesitation to move to a new type of product, unless aptamers offer verifiably significant improvements on current technologies that warrant substitution of antibodies in some current assay formats. In this review, different types of electrochemical aptamer-based biosensors have been discussed. Although the optical and mass-sensitive aptasensors have been the most commonly described in the literature, electrochemical transducers have enormous potential and offer simple, rapid, cost-effective and easy to miniaturize sensing in many diagnostic fields. Emerging nanomaterials have also brought new possibilities for developing novel ultrasensitive electrochemical aptasensors.

## References

- Baker BR, Lai RY, Wood MCS, Doctor EH, Heeger AJ, Plaxco KW (2006) An electronic, aptamer-based small-molecule sensor for the rapid label-free detection of cocaine in adulterated samples and biological fluids. **J Am Chem Soc** 128, 3138-3139.
- Balamurugan S, Obubuafo A, Soper SA, Spivak DA (2008) Surface immobilization methods for aptamer diagnostic applications. **Anal Bioanal Chem** 390, 1009-1021.
- Bang GS, Cho S, Kim BG (2005) A novel electrochemical detection method for aptamer biosensors. **Biosens Bioelec** 21, 863-870.
- Berezovski M, Drabovich A, Krylova SM, Musheev M, Okhonin V, Petrov A, Krylov SN (2005) Nonequilibrium Capillary Electrophoresis of Equilibrium Mixtures: A Universal Tool for Development of Aptamers. **J Am Chem Soc** 127, 3165-3171.
- Bini A, Minunni M, Tombelli S, Centi S, Mascini M (2007) Analytical performances of aptamer-based sensing for thrombin detection. **Anal Chem** 79, 3016-3019.
- Centi S, Messina G, Tombelli S, Palchetti I, Mascini M (2008) Different approaches for the detection of thrombin by an electrochemical aptamer-based assay coupled to magnetic beads. **Biosens Bioelec** 23, 1602-1609.
- Cho EJ, Collett JR, Szafranska AE, Ellington AD (2006) Optimization of aptamer microarray technology for multiple protein targets. **Anal Chim Acta** 564, 82-90.
- Cho S, Kim JE, Lee BR, Kim JH, Kim BG (2005) Bis-aptazyme sensors for hepatitis C virus replicase and helicase without blank signal. **Nucleic Acids Res** 33, e177.
- Choi JH, Chen KH, Strano MS (2006) Aptamer-Capped Nanocrystal Quantum Dots: A New Method for Label-Free Protein Detection. **J Am Chem Soc** 128, 15584-15585.
- De-los-Santos-Alvarez N, Lobo-Castañón MJ, Miranda-Ordieres AJ, Tuñón-Blanco P (2008) Aptamers as recognition elements for label-free analytical devices. **Trends Anal Chem** 27, 437-446.
- Drabovich A, Berezovski M, Krylov SN (2005) Selection of Smart Aptamers by Equilibrium Capillary Electrophoresis of Equilibrium Mixtures (ECEEM). **J Am Chem Soc** 127, 11224-11225.
- Ellington AD, Szostak JW (1990) In vitro selection of RNA molecules that bind specific ligands. **Nature** 346, 818-822.
- Ferreira CSM, Matthews CS, Missailidis S (2006) DNA aptamers that bind to MUC1 tumour marker: Design and characterization of MUC1-binding single stranded DNA aptamers. **Tumor Biol** 27, 289-301.
- Ferreira CSM, Papamichael K, Guilbault G, Schwarzacher T, Garipey J, Missailidis S (2008) Design of aptamer-antibody sandwich ELISA for early tumour diagnosis. **Anal Bioanal Chem** 390, 1039-1050.
- Fraundorf C, Jaschke A (2001) Detection of small organic analytes by fluorescing molecular switches. **Bioorg Med Chem** 9, 2521-2524.
- Fredriksson S, Gullberg M, Jarvius J, Olsson C, Pietras K, Gustafsdottir SM, Ostman A, Landegren U (2002) Protein detection using proximity-dependent DNA ligation assays. **Nature Biotechnol** 20, 473-477.
- Hamula CLA, Guthrie JW, Zhang H, Li XF, Le XC (2006) Selection and analytical applications of aptamers. **Trends Anal Chem** 25, 681-691.
- Hansen JA, Wang J, Kawde AN, Xiang Y, Gothelf KV, Collins G (2006) Quantum-dot/aptamer-based ultrasensitive multi-analyte electrochemical biosensor. **J Am Chem Soc** 128, 2228-2229.
- Heidenreich O, Eckstein F (1992) Hammerhead ribozyme-mediated cleavage of the long terminal repeat RNA of human immunodeficiency virus type 1. **J Biol Chem** 267, 1904-1909.
- Hianik T, Ostatna V, Sonlajtnerova M, Grman I (2007) Influence of ionic strength, pH and aptamer configuration for binding affinity to thrombin. **Bioelectrochemistry** 70, 127-133.
- Ikanovic M, Rudzinski WE, Bruno JG, Allman A, Carrillo MP, Dwarakanath S, Bhaahdigadi S, Rao P, Kiel JL, Andrews CJ (2007) Fluorescence Assay Based on Aptamer-Quantum Dot Binding to Bacillus thuringiensis Spores. **J Fluoresc** 17, 193-199.
- Ikebukuro K, Kiyohara C, Sode K (2005) Novel electrochemical sensor system for protein using the aptamers in sandwich manner. **Biosens Bioelec** 20, 2168-2172.
- Ikebukuro K, Okumura Y, Sumikura K, Karube I (2005) A novel method of screening thrombin-inhibiting DNA aptamers using an evolution-mimicking algorithm. **Nucleic Acids Res** 33, e108.
- Kang Y, Feng KJ, Chen JW, Jiang JH, Shen GL, Yu RQ (2008) Electrochemical detection of thrombin by sandwich approach using antibody and aptamer. **Bioelectrochemistry** 73, 76-81.
- Khan H, Missailidis S (2008) Aptamers in oncology: a diagnostic perspective. **Gene Ther Mol Biol** 12, 111-128.
- Kim YS, Jung HS, Matsuura T, Lee HY, Kawai T, Gu MB (2007) Electrochemical detection of 17beta-estradiol using DNA aptamer immobilized gold electrode chip. **Biosens Bioelec** 22, 2525-2531.
- Kirby R, Cho EJ, Gehrke B, Bayer T, Park YS, Neikirk DP, McDevitt JT, Ellington AD (2004) Aptamer-based sensor arrays for the detection and quantification of proteins. **Anal Chem** 76, 4066-4075.
- Kusser W (2000) Chemically modified nucleic acid aptamers for in vitro selections: evolving evolution. **Rev Mol Biotechnol** 74, 27-38.
- Lai RY, Plaxco KW, Heeger AJ (2007) Aptamer-based electrochemical detection of picomolar platelet-derived growth factor directly in blood serum. **Anal Chem** 79, 229-233.

- Levy M, Cater SF, Ellington AD (2005) Quantum-Dot Aptamer Beacons for the Detection of Proteins. **ChemBioChem** 6, 2163-2166.
- Li X, Qi H, Shen L, Gao Q, Zhang C (2008) Electrochemical Aptasensor for the Determination of Cocaine Incorporating Gold Nanoparticles Modification. **Electroanalysis** 20, 1475-1482.
- Liao W, Cui XT (2007) Reagentless aptamer based impedance biosensor for monitoring a neuro-inflammatory cytokine PDGF. **Biosens Bioelec** 23, 218-224.
- Liu J, Lee JH, Lu Y (2007) Quantum Dot Encoding of Aptamer-Linked Nanostructures for One-Pot Simultaneous Detection of Multiple Analytes. **Anal Chem** 79, 4120-4125.
- Liu J, Lu Y (2004) Adenosine-dependent assembly of aptazyme-functionalized gold nanoparticles and its application as a colorimetric biosensor. **Anal Chem** 76, 1627-1632.
- Liu J, Lu Y (2006) Fast colorimetric sensing of adenosine and cocaine based on a general sensor design involving aptamers and nanoparticles. **Angew Chem Int Ed** 45, 90-94.
- Lu Y, Zhu N, Yu P, Mao L (2008) Aptamer-based electrochemical sensors that are not based on the target binding-induced conformational change of aptamers. **Analyst** 133, 1256-1260.
- Luzi E, Minunni M, Tombelli S, Mascini M (2003) New trends in affinity sensing: aptamers for ligand binding. **Trends Anal Chem** 22, 810-818.
- Min K, Cho M, Han SY, Shim YB, Ku J, Ban C (2008) A simple and direct electrochemical detection of interferogamma using its RNA and DNA aptamers. **Biosens Bioelec** 23, 1819-1824.
- Mir M, Vreeke M, Katakis I (2006) Different strategies to develop an electrochemical thrombin aptasensor. **Electrochem Comm** 8, 505-511.
- Mir M, Vreeke M, Katakis I (2008) Ultrasensitive detection based on an aptamer beacon electron transfer chain. **Electrochem Comm** 10, 1533-1536.
- Missailidis S (2008) Combinatorial Approach to Anticancer Drug Design, In-Anticancer Therapeutics (Eds: S. Missailidis), Wiley & Sons Ltd.
- Ogawa A, Maeda M (2007) Aptazyme-based riboswitches as label-free and detector-free sensors for cofactors. **Bioorg Med Chem Letters** 17, 3156-3160.
- Ozaki H, Nishihira A, Wakabayashi M, Kuwahara M, Sawai H (2006) Biomolecular sensor based on fluorescence-labeled aptamer. **Bioorg Med Chem Letters** 16, 4381-4384.
- Papamichael KI, Kreuzer MP, Guilbault GG (2007) Viability of allergy (IgE) detection using an alternative aptamer receptor and electrochemical means. **Sens Actuat B-Chem** 121, 178-186.
- Pieken W, Olsen DB, Benseler F, Aurup H, Eckstein HF (1991) Kinetic characterization of ribonuclease-resistant 2'-modified hammerhead ribozymes. **Science** 253, 314-317.
- Polsky R, Gill R, Willner I (2006) Nucleic Acid-Functionalized Pt Nanoparticles: Catalytic Labels for the Amplified Electrochemical Detection of Biomolecules. **Anal Chem** 78, 2268-2271.
- Potyrailo RA, Conrad RC, Ellington AD, Hieftje GM (1998) Adapting selected nucleic acid ligands (aptamers) to biosensors. **Anal Chem** 70, 3419-3425.
- Song S, Wang L, Li J, Zhao J, Fan C (2008) Aptamer-based biosensors. **Trends Anal Chem** 27, 108-117.
- Stojanovic MN, Kolpashchikov DM (2004) Modular aptameric sensors. **J Am Chem Soc** 126, 9266-9270.
- Stojanovic MN, Landry DW (2002) Aptamer-based colorimetric probe for cocaine. **J Am Chem Soc** 124, 9678-9679.
- Stojanovic MN, Prada PD, Landry DW (2001) Aptamer-based folding fluorescent sensor for cocaine. **J Am Chem Soc** 123, 4928-4931.
- Suprun E, Shumyantseva V, Bulko T, Rachmetova S, Rad'ko S, Bodeev N, Archakov A (2008) Au-nanoparticles as an electrochemical sensing platform for aptamer-thrombin interaction. **Biosens Bioelec** 24, 831-836.
- Tombelli S, Minunni M, Mascini M (2005) Analytical applications of aptamers. **Biosens Bioelec** 20, 2424-2434.
- Tombelli S, Minunni M, Mascini M (2007) Aptamers- based assays for diagnostics, environmental and food analysis. **Biomol Eng** 24, 191-200.
- Tuerk C, Gold L (1990) Systematic evolution of ligands by exponential enrichment - RNA ligands to bacteriophage-T4 DNA-polymerase. **Science** 249, 505-510.
- Velasco-Garcia MN, Mottram TT (2003) Biosensor Technology addressing Agricultural Problems **Biosys Eng** 84, 1-12.
- Wang J (2003) Nanoparticle-based electrochemical DNA detection. **Anal Chim Acta** 500, 247-257.
- Winters-Hilt S (2006) Nanopore Detector based analysis of single-molecule conformational kinetics and binding interactions. **BMC Bioinformatics** 7, S21. [www.lsciences.com](http://www.lsciences.com), accessed January 2009. [www.somalagic.com](http://www.somalagic.com), accessed January 2009.
- Xiao Y, Lubin AA, Heeger AJ, Plaxco KW (2005) Label-free electronic detection of thrombin in blood serum by using an aptamer-based sensor. **Angew Chem Int Ed** 44, 5456-5459.
- Yamana K, Ohtani Y, Nakano H, Saito I (2003) Bis-pyrene labeled DNA aptamer as an intelligent fluorescent biosensor. **Bioorg Med Chem Letters** 13, 4329-4331.
- Yoshida W, Sode K, Idebukuro K (2006b) Homogeneous DNA sensing using enzyme-inhibiting DNA aptamers. **Biochem Biophys Res Comm** 348, 245-252.
- Yoshida W, Sode K, Idebukuro K (2006a) Aptameric Enzyme Subunit for Biosensing Based on Enzymatic Activity Measurement. **Anal Chem** 78, 3296-3303.
- Zheng F, Wu ZS, Zhang SB, Guo MM, Chen CR, Shen GL, Yu RQ (2008) Aptamer-based Electrochemical Biosensors for Highly Selective and Quantitative Detection of Adenosine. **Chem Res Chinese Universities** 24, 138-142.
- Zheng J, Feng W, Lin L, Zhang F, Cheng G, He P, Fang Y (2007) A new amplification strategy for ultrasensitive electrochemical aptasensor with network-like thiocyanuric acid/gold nanoparticles. **Biosens Bioelec** 23, 341-347.
- Zuo X, Song S, Zhang J, Pan D, Wang L, Fan C (2007) A target-responsive electrochemical aptamer switch (TREAS) for reagentless detection of nanomolar ATP. **J Am Chem Soc** 129, 1042-1043.



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