

Developing Trends in Aptamer-Based Biosensor Devices and Their Applications

Scott MacKay, David Wishart, James Z. Xing, and Jie Chen

Abstract—Aptamers are, in general, easier to produce, easier to store and are able to bind to a wider variety of targets than antibodies. For these reasons, aptamers are gaining increasing popularity in environmental monitoring as well as disease detection and disease management applications. This review article examines the research and design of RNA and DNA aptamer based biosensor systems and applications as well as their potential for integration in effective biosensor devices. As single stranded DNA or RNA molecules that can bind to specific targets, aptamers are well suited for biomolecular recognition and sensing applications. Beyond being able to be designed for a near endless number of specific targets, aptamers can also be made which change their conformation in a predictable and consistent way upon binding. This can lead to many unique and effective detection methods using a variety of optical and electrochemical means.

Index Terms—Antibody, aptamer, biosensor, carbon nanotube, electrochemical detection, mass based detection, optical detection, RNA/DNA, SELEX.

I. INTRODUCTION

ACCURATELY detecting, identifying and quantifying biomolecules and other biological substances is of great importance in many fields of life science. For instance, the detection of bacteria, viruses and other pathogens is critical for environmental and food safety monitoring. The detection of abnormal proteins or abnormal levels of proteins, such as troponin I or C-reactive protein is critical to diagnosing a number of diseases [1]. Likewise the measurement of metabolites such as glucose or creatinine is important for the monitoring

of organ function and metabolic stability [2], [3]. Bacteria, proteins and metabolites are examples of biological materials or biopolymers that need to be routinely detected. While many biological detection methods utilize large instruments such as chemical analyzers, mass spectrometers or nuclear magnetic resonance (NMR) spectrometers, there are a growing number of biological and chemical detection methods that rely on smaller biosensors [4], [5]. A biosensor is a device that can be used for the detection of a molecule, which combines a “soft” biological component with a “hard” physicochemical detector. The biological component is usually the recognition element, the part of the biosensor which specifically recognizes the presence of the target molecule; while the physicochemical detection element is the part of the biosensor which translates the recognition of the target into a measurable and reportable signal [6]. There are many variations of recognition and detection elements leading to a number of different biosensor designs, each of which suited to specific targets and applications [7]–[15]. Perhaps the most widespread biosensor is blood glucose monitor, a device that is used on a daily basis by millions of diabetics worldwide [2], [16]. The modern glucose biosensor uses the enzyme glucose oxidase to break down blood glucose leading to the release of electrons and a detectable current [17]. In this case, the electrode is the detection element and the enzyme is the biological recognition element. Enzymes need not be the only possible bio-recognition element for biosensors, in fact the biological recognition elements of biosensors can vary from enzymes to antibodies to peptides to aptamers.

Aptamers (from the Latin aptus, meaning fit, and Greek meros, meaning region) are literally polymers designed to fit specific substrates. Aptamers can be composed of nucleotides or amino acids, although most often the term is reserved for RNA or DNA oligonucleotides [18]. Aptamers have proven to be an effective tool in the creation of novel, accurate and very specific biosensors for a variety of applications ranging from food and environmental testing [19]–[22], to toxin detection [22], to protein monitoring [12], [13], [23], [24], to a range of metabolomic and chemical sensing applications [11], [25], [26]. Aptamer-based sensors take advantage of the ability of aptamers to bind strongly to specific targets as well as their ability to undergo substantial conformational changes upon binding [15], [27], [28]. There are many detection methods which utilize aptamers, including fluorescent and optical detection [7], [8], [10], [21], [28], electrochemical and electrical detection [11]–[13], [19], [25], [29]–[31], as well as mechanical (mass-based) detection [9], [24].

In some respects, aptamers are very similar to antibodies as they can both bind to biomolecules with great specificity.

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Although antibodies have also been used in the creation of biosensors, aptamers present detection opportunities that were not previously available through other biosensing modalities due to their size (being smaller than antibodies), stability, and structure switching capabilities [32]. This review provides a brief overview of aptamers, their associated detection modalities, their applications in biosensing, and their potential for integration with other design elements, such as microfabricated devices, CMOS electronics and microfluidics, for the creation of effective complete biosensors.

As with similar research in detecting biomolecules, the final goal of much aptamer-based biodetection research is the eventual creation of complete biosensing systems which utilize aptamers for the recognition of specific biological molecules. It therefore becomes necessary to not only consider the properties of aptamers to fit target biomolecules, but also how they can best be incorporated into biosensor systems and the other design aspects that must be considered in creating a biosensor that can take advantage of many of the favorable properties aptamers provide in biosensing applications.

II. BACKGROUND

Aptamers are double-stranded DNA or single-stranded RNA/DNA molecules that bind specific molecular targets. The first aptamers were discovered or developed independently by two different laboratories in 1990. In particular, Larry Gold at the University of Colorado in Boulder, designed aptamers to bind to T4 DNA polymerase [33] while Jack Szostak at the Massachusetts General Hospital in Boston, created RNA aptamers to bind to various organic dyes [34]. It was Szostak who actually coined the term ‘aptamer’. Depending on their sequence, aptamers can be made to bind a wide variety of targets ranging from metal ions, to metabolites, to specific proteins, to subcellular organelles—even to full sized cells [7], [8], [27], [34]. Aptamers are similar to antibodies in their selective binding, but also offer a number of distinct advantages over antibodies. Aptamers are, in general, easier to produce, easier to store and maintain and are able to bind to a wider variety of targets than antibodies [9]. While most aptamers in use today are synthetic or “designer” molecules, natural aptamers also exist. These naturally occurring aptamers or “riboswitches” were first discovered in 2002 and were found to possess similar molecular recognition properties to synthetic aptamers [35]. A riboswitch is a part of an mRNA molecule that directly binds to a small target molecule, usually a metabolite, to change the gene’s activity. Riboswitches may also be RNA enzymes (ribozymes) that cleave themselves in the presence of a small molecule metabolite [36]. Many of the earliest riboswitches were found in the 5′ untranslated regions of mRNAs. To date, most known riboswitches have been found in viruses and eubacteria, but they have also been discovered in plants and certain fungi [36]. The first human riboswitch was identified in 2009 in human vascular endothelial growth factor (VEGF) [37].

The most widely used technique for the production of synthetic aptamers is SELEX [7], [33] (Systematic Evolution of Ligands by EXponential enrichment). The process begins with a random set of single stranded nucleic acids (ssRNA or ssDNA). Next, a sample of the desired target is added to the nucleic

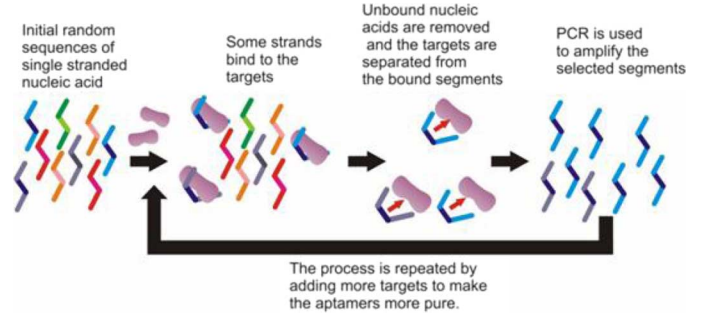


Fig. 1. A simplified process flow for the SELEX method of making aptamers.

acids where some of those random sequences bind with the targets (approximately one strand in every 10^{10} strands will bind [7]). The unbound targets and nucleic acids are then removed, leaving only the desired strands. The bound targets are then removed and the selected strands are amplified using PCR (polymerase chain reaction). This process is repeated five to six times using the selected strands as the starting nucleic acids in order to further refine the aptamers. Unlike the production of antibodies or enzymes, this is an entirely *in-vitro* process. This means that aptamers can be easily made to target molecules that are toxic to cells or targets which would present complications to *in-vivo* production methods [9]. Although first developed for protein targets, the SELEX technique can be used for potentially any target molecule [33].

III. DETECTION METHODS

As mentioned previously, there are several different methods by which the presence of a target can be reported by aptamers. These generally fall into three categories: optical detection, electrical and electrochemical detection, and mechanical detection. Although all of these techniques have been and are currently being studied, many researchers prefer electrochemical techniques as they tend to be simpler, more accurate, more sensitive and generally cheaper than other detection methods [30].

A. Optical Detection

Although the individual techniques can vary, most optical detection methods take advantage of the conformational changes occurring in aptamers to either generate or quench fluorescence. Optical detection methods have significant appeal because of their ease of detection and their exquisite sensitivity [8], [10], [38].

The simplest route to achieve optical detection in aptamer-based biosensors is to chemically attach a fluorophore to a specific site on the aptamer. The conformational change induced in the aptamer by the target molecule almost always changes the interactions between the fluorescent nucleotides of the aptamer and the fluorophore, causing a change in the fluorescent output [10], [39]. Therefore, if a change in fluorescence is detected, this indicates that the target molecule is present. This particular type of detection has been shown to detect ATP at concentrations as low as 1 mM [39].

Another optical detection method that can be used is to chemically attach both a fluorophore and a quencher molecule to the

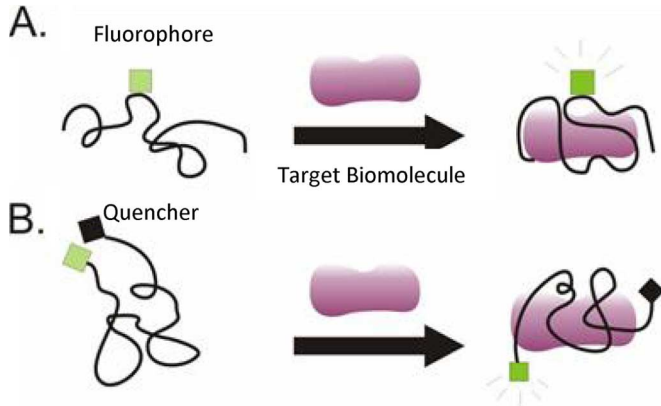


Fig. 2. (a) An optical detection method where the conformational change in the aptamer upon binding changes the fluorescence produced by the fluorophore [7]. (b) In the unbound state, the quencher (black square) is in close proximity to the fluorophore (green square), but when the aptamer is bound to the target, they move apart and fluorescence can be measured [7], [40].

aptamer. These molecules are placed in such a way that in the aptamer's unbound state the quencher is close enough to the fluorophore to eliminate any fluorescence from being generated. However, when the aptamer is bound to the target molecule, the quencher and fluorophore are separated, and fluorescence can be detected [10]. The reverse is also possible, where fluorescence is detected when the aptamer is ligand-free and is quenched when the ligand is bound [10]. The preparation of these double labeled aptamers is somewhat more complex than just using one fluorophore, but double-labeled aptamers also generate a greater change in fluorescence, making detection easier. This fluorophore-quencher aptamer biosensor has been used to detect thrombin with a detection limit of about 0.3 nM [40]. Another similar technique is to use a second fluorophore rather than a quencher [10]. In this case, the change in proximity of the fluorophores causes fluorescence resonance energy transfer (FRET), which can be measured [6], [10]. Carbon nanotubes [41] and graphene [42], [43] have also been utilized as quenchers, blocking fluorescence from fluorophores on aptamers when they are not bound to their target ligand. Graphene quenching has been used to detect oxytetracycline with a limit of detection of 10 nM [43]. When bound to the target ligand, the conformational change in the aptamer makes quenching from the carbon nanotube or graphene impossible [41]–[43]. Other variations on this design use separate strands of DNA labeled with quenchers or fluorophores [38], [44]. The quencher strand and fluorophore labeled aptamer are complementary enough to bind when no target is present, thus quenching the fluorescence, but when the target is present, the favorable interaction is for the aptamer to bind to the target, thus preventing quenching [45].

Optical detection techniques without the use of fluorophores have also been explored. These non-fluorescent biosensors are created by attaching aptamers to gold nanoparticles [7]. When the target biomolecules are bound to the aptamers they prevent the gold nanoparticles from aggregating in solution (through electrostatic repulsion). Conversely, without the target biomolecules present, the gold nanoparticles will aggregate. By measuring the optical density of the gold nanoparticle solution, the degree of aggregation can be determined and therefore also

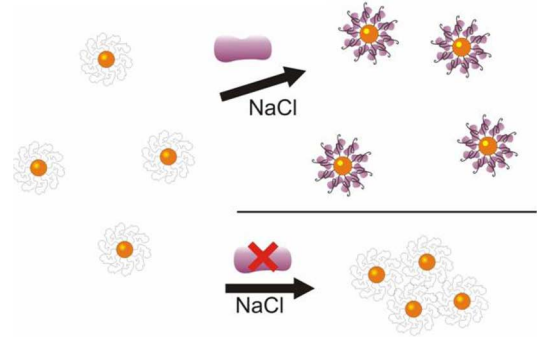


Fig. 3. In the presence of the target, aptamer coated gold nanoparticles do not aggregate due to electrostatic repulsion. Without the target, over time the gold nanoparticles will aggregate together in the solution. The difference between these two cases can be detected by measuring optical density [7].

the presence of the target molecule can be ascertained [7], [46]. This technique has been shown to detect as little as 8 nM of mucin 1 peptide [7]. The reverse is also possible, where the addition of the target ligand (such as adenosine [47]) causes the aggregation of gold nanoparticles. Other groups have described a technique using unlabeled nanoparticles, where aptamers which do not bind to the target molecule instead prevent the nanoparticles from aggregating [48].

Another optical detection technique involving nanoparticles employs aptamers attached to quantum dots [14]. Rather than measuring particle aggregation, this technique relies on the fact that quantum dots are also fluorescent and therefore can undergo FRET. The researchers who developed this system designed it so that when no target biomolecule (in this case ATP [14]) is present, strands of DNA labeled with a fluorophore (Cy5 [14]) attach to the aptamer. FRET occurs between the quantum dot and the fluorophore (with the label fluorophore being the acceptor and the quantum dot being the donor) and certain emission wavelengths are detectable. If the target molecule is present, the labeled DNA cannot bind, resulting in no FRET and therefore a different measured emission wavelength [14]. This method has been reported to have a limit of detection of 0.024 mM (for ATP detection) [14].

Optical techniques often depend on labeling aptamers or other components of the sensor with fluorescent molecules or quenchers, which is a relatively complex and costly process [8], [10]. The addition of these extra molecules to the aptamers can also cause changes in the affinity or stability of the aptamers [8]. These issues have led to increased efforts to create label-free aptamer based biosensors.

B. Electrical and Electrochemical Detection

In general, electrochemical detection for biosensors involves detecting changes in the electrical properties of the sensor system (usually electrical impedance) caused by aptamers binding to their specific targets. There are several different techniques based on what electrical properties are measured, how they are measured and what specifically causes the change when binding occurs.

Electrochemical detection methods often involve first immobilizing one end of an aptamer on a solid surface, and then measuring the electrical properties of that surface. A very commonly

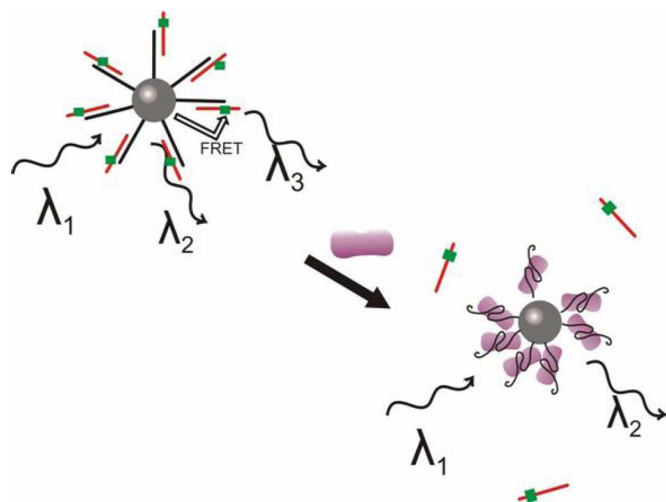


Fig. 4. Without the target present, the single strands of DNA with attached fluorophores (red lines with attached green squares in this figure) attach to the unbound aptamers on the quantum dots. When the excitation wavelength is added (λ_1), FRET occurs between the quantum dot and the fluorophore, producing two emission wavelengths (λ_2 and λ_3). With the target present, the affinity of the aptamer for the target is greater than for the second DNA sequence, thus the aptamers bind with the targets only, no FRET occurs and there is only one emission wavelength [14].

used material for these surfaces is gold [29] as it is generally nonreactive, has well established electrical properties and covalent linkage to gold is easily accomplished through the use of a thiol bond [49].

In some cases, a change in the conductivity of a gold surface is caused through the use of a redox label [11], [29]. In these applications, a redox label, such as methylene blue [11], is attached to the far end of the aptamer (with the other end of the aptamer connected to a gold electrode). Upon binding with the target biomolecule, the aptamer's 3D structure changes in such a way that the end attached to the redox agent is brought into close proximity with the gold surface. When the redox label and the gold surface are brought together, electron transfer occurs as the redox agent is either reduced or oxidized (depending on the chosen redox label). This electron transfer causes a change in the electrical properties that can be measured using voltammetry [11], [19], [29]. It is also possible to have aptamers which in their unbound state have the redox label close to the surface and the redox agent is moved away from the surface after binding occurs. This technique has been used previously to detect a variety of biomolecules such as interferon gamma [11], ochratoxin A [19], lysozyme [50] and thrombin [29]. A number of different redox labels have been studied for this application including methylene blue [11], [29], $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ [25], [50] and juglone [30]. Using this technique, thrombin has been measured with a limit of detection of 11 nM [29], interferon gamma has been measured with a limit of detection of 0.06 nM [11], and ochratoxin A and lysozyme have been measured with a limit of detection of 0.07 nM [19].

A similar technique that has been studied is measuring impedance spectra rather than redox current (using voltammetry) [12], [31]. Again, aptamers are attached to a gold surface, however, no extra redox modification is necessary as the binding of the target molecule (usually a protein) is enough

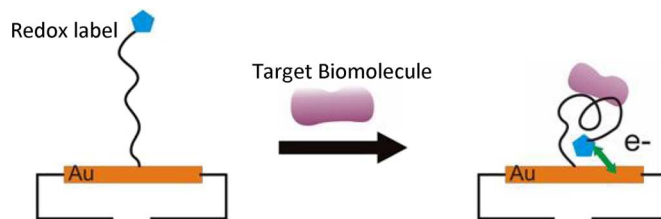


Fig. 5. Electrochemical detection using a redox label. Without the target present, the conformation of the aptamer keeps the redox label (blue pentagon) too far from the gold surface for a reaction to take place. With the aptamer bound to the target, the conformation allows a redox reaction to take place (and therefore an exchange of electrons and a measurable change in the current) [29]. The electrical contacts attached to the gold surface are connected to measuring equipment that varies depending on the particular method used.

to cause a measurable change in the impedance spectrum [31]. Thrombin has been detected using this technique with a limit of detection as low as 0.1 nM [31]. This arrangement is particularly advantageous as it is simpler than the similar redox label techniques and it does not rely on specific conformational changes in the aptamers (requiring only the binding itself). However, impedance measurements can be easily influenced by other factors in the system (such as temperature or ion concentrations), leading to difficulty in obtaining accurate, reliable results [11].

1) Nanoparticle Enhanced Electrical Detection: Just as with optical detection methods, nanoparticles have been used to enhance electrochemical detection methods as well. Specifically, gold nanoparticles are particularly appealing as they can be chemically modified for a wide variety of applications [51], [52]. A number of techniques have been developed which use nanoparticles to improve upon and as the basis for electrochemical sensor designs [47], [53]. One such improvement has been achieved by modifying gold surfaces and electrodes by adding gold nanoparticles [26], [47], [54], [55]. The addition of gold nanoparticles keeps the same electrical properties as simple gold films but greatly increases the surface area and therefore more aptamers can be bound to the interface, increasing sensor sensitivity [52], [54], [55]. Different techniques have been used to attach these nanoparticles, including physical adsorption [54], electrodeposition [54] and chemical linkage [55]. Further interface modifications have also been studied, including adding electropolymerized tyramine and cystamine in addition to gold nanoparticles to amplify electrochemical currents by up to 300% [56].

Gold nanoparticles have also been studied extensively as probes in electrochemical sensors. As probes, nanoparticles are coated with aptamers that can then bind to targets already immobilized on the electrode surface via another recognition element. This recognition element can be another aptamer or an antibody in a sandwich configuration [15], [47]. The probe is then able to amplify the measured signal on the electrode due to other elements attached to the nanoparticles such as redox labels or charged molecules. Using gold nanoparticles as probe vectors rather than just labeled aptamers (as discussed previously) is advantageous as a large number of reporter molecules (like redox labels) can be attached to each nanoparticle, increasing the measured signal. Furthermore, a wider variety of

reporter molecules can be used with nanoparticles than with aptamers alone [47]. Other forms of signal enhancement are also possible using gold nanoparticles for electrochemical detection. Using a combination of enhancement effects from redox labels and gold nanoparticle enlargement Deng *et al.* created a biosensor which can detect thrombin with a limit of detection as low as 100 fM [57].

Our own group is currently focused on creating an impedance-based sensor using aptamers as well as signal enhancement using gold nanoparticles. Like the above mentioned methods, the nanoparticles are used as probes to enhance the measured signal. However, rather than serving as a redox label, the gold nanoparticles are coated with PEG (polyethylene glycol) so that when a slightly basic (pH 8.0) solution is added, they become charged. These charges (and therefore whether or not the particles are bound to the gold electrode) can then be detected using a simple conductivity measurement. The other novel aspect of our sensor lies in the way that the aptamer, gold surface and nanoparticle probe connect to one another. Rather than a sandwich configuration which requires two aptamers and specific target types to function, this sensor takes advantage of the conformational changes that take place when an aptamer binds its target ligand. In this system, short DNA sequences are first attached to the gold electrode and the PEG coated gold nanoparticles (via disulfide bonds). Aptamers are used which are modified such that they have short sequences on their ends which are complementary to the segments on the electrode (at one end) and the nanoparticles (at the other end). When no ligand is present, one of these ends is blocked, thus preventing the attachment of the probe and electrode, however when the aptamer binds with the ligand, the conformation of the aptamer changes so that both ends are free and the electrode and probe can be linked. As of now, initial proof-of-concept experiments and preliminary designs have been completed, both of which look promising. If successful, this sensor system will be very versatile, limited only by what aptamers can be made with the required conformational changes. Of course it will still have some of the same target limitations that other impedance-based systems have. The design is also considerably simpler than other methods, requiring only a single impedance measurement, as opposed to the relatively expensive and time consuming impedance spectroscopy. While this does make design and implementation simpler, it may also limit the sensitivity of the final device.

2) *Carbon Nanotube Based Electrical Sensors:* Aptamers have been linked to carbon nanotube field effect transistors (CNT-FET) to create electrochemical biosensors [13], [58]. In this sort of application aptamers are covalently linked to carbon nanotubes which span between the source and drain of CNT-FET devices. When the target biomolecule binds to the attached aptamers there is a measureable decrease in source-drain current [13], [58]. Other changes in the electrical properties of these CNT-FET biosensors are also detectable in the presence of small concentrations of the target molecule as well [13]. Creating aptamers as the source of target recognition with this detection technique is crucial as they remain stable after binding with the carbon nanotubes and are small enough that binding influences the electrical properties of the sensor

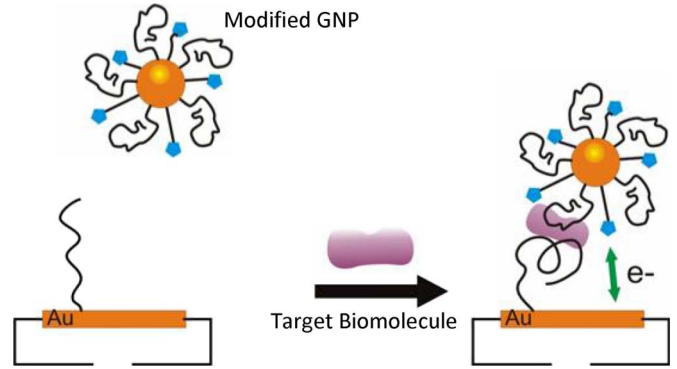


Fig. 6. A simple diagram of a biosensor which utilizes gold nanoparticles as redox probes. The target, electrode aptamer and gold nanoparticle aptamer bind together in a sandwich configuration (with the different aptamers binding to different sections of the target). The binding brings the redox labels (blue pentagons) on the nanoparticles close enough to the electrode to result in electron transfer [47], [57]. The electrical contacts attached to the gold surface are connected to measuring equipment that varies depending on the particular method used.

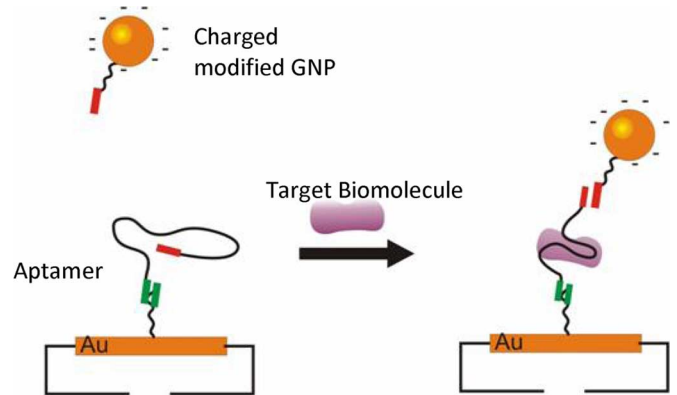


Fig. 7. Diagram representation of our own group's proposed impedance based sensor. Rather than a sandwich configuration, signal enhancing gold nanoparticles attach to a section of the aptamer which is freed through the conformational changes induced by binding with the target. The electrical contacts on the gold surface are connected to a meter which measures electrical impedance.

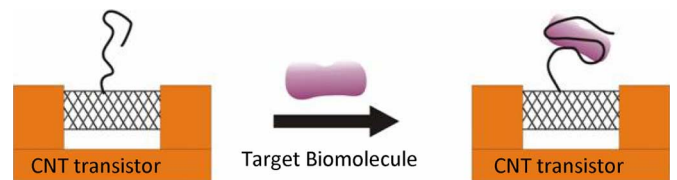


Fig. 8. In the CNT transistor technique, the aptamers are bound to the carbon nanotube in a CNT transistor. When target binding occurs there are measurable electrical changes in the transistor [13].

(binding occurs within the electrical screening depth of the biosensor system) [13]. CNT-FET aptamer sensors have been reported to detect IgE at concentrations as low as 250 pM [13]. A similar method presented by Liu *et al.* places the detecting aptamer in a gap in between two CNTs and is capable of single-molecule detection of thrombin [59].

Although electrochemical detection offers many advantages over other detection techniques for aptamer-based biosensors,



Fig. 9. Basis of cantilever biosensors. Microfabricated silicon cantilevers are coated in a thin gold layer to which aptamers are attached. Target binding causes a deflection in the cantilever, which can be detected optically.

there are noteworthy limitations. In particular, the target molecule must not generate a measurable effect on the electrical properties of the sensor (e.g., cause extra free charges or change the conductivity of the system) and must not react independently with any redox agents used. This prevents testing for many charged entities and some others. In addition to this, any other background species in any tested solutions must also not have an effect on electrical properties.

C. Mass Based Detection

Quartz crystal microbalances (QCMs) have long been used as the basis of acoustic detection [60]. By attaching aptamers to quartz crystals (using a thin layer of gold on the quartz), it is possible to measure the changes in the vibrational frequency of these crystals as a way of determining if the target molecule has been attached to the aptamers [9]. This technique was first employed using antibodies as the recognition elements. Unlike the previously described detection techniques, QCMs do not rely on any conformational change in the aptamers, just the binding itself.

Even though antibodies can also be used as the recognition element in QCM based biosensors, aptamers have been shown to have several distinct advantages. Aptamer based QCM biosensors are more sensitive, have a lower limit of detection (as low as 50 pM for IgE detection), are more stable and more accurate than antibody-based QCM sensors [9]. However, the applications of most QCM biosensors are somewhat limited as, they must operate in gaseous environments, in order to resonate properly. In some cases it is possible to first expose the sensor to a test solution, then remove the solution (and wash the sensor) to take a final measurement [61].

A similar approach has been studied using microfabricated cantilevers. With either antibodies or aptamers attached to these cantilevers, the presence of target biomolecules can be detected based on cantilever bending arising from the extra mass of the target ligands [62]. This kind of stress-induced bending can be detected using optical methods such as measuring the reflection of laser light incident on the end of the cantilevers [24]. These systems have been implemented successfully in liquid environments, although the extra stresses induced in the cantilevers from elements in the testing solution can be problematic [24]. These problems can be addressed however, through the use of reference cantilevers in the system (microfabricated cantilevers without attached aptamers serving as a removable background measurement) [24]. Using this method, the detection of TAQ DNA polymerase at concentrations as low as 50 pM has been reported [24].

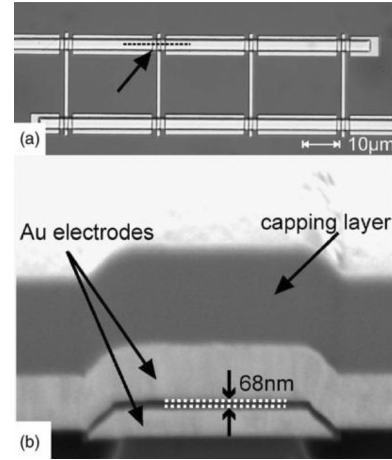


Fig. 10. (a) Optical microscope image of the microfabricated aptamer-based biosensor electrodes created by Löhndorf *et al.* (b) SEM image of a single electrode pair, showing the gap between the pair [64] (reprinted with the permission of Applied Physics Letters).

IV. INTEGRATED SENSOR MICROSYSTEMS

A. Nanotechnology and Microfabrication

The end goal of the aptamer-based detection methods discussed, regardless of the individual intended applications, is to create a complete functional biosensor system using that particular detection methodology. To achieve this goal, the individual principles of using aptamers for detection must be integrated into an appropriate system. These systems incorporate elements such as microfabrication techniques and microfluidic systems to create more efficient and effective biosensors.

As with other applications, microfabrication and nanofabrication techniques can be used to create highly effective, efficient and inexpensive biosensors. There has been a trend in recent research to apply these manufacturing techniques for aptamer-based biosensors specifically. The use of microfabricated components can be potentially useful for most types of aptamer-based biosensor techniques including optical, mass-based and electrochemical detection [63]. Microfabricated electrodes have been used as platforms for electrical and electrochemical aptamer-based biosensors [63], [64] as well as a variety of other biosensor systems [23], [65]. Microfabrication techniques are used to pattern thin metal layers deposited on substrates, often gold, to create small, sensitive electrodes for these applications. Arrays of electrodes can be patterned onto single substrates for higher throughput biosensors as well as for increased sensitivity and the potential for detecting multiple targets simultaneously from a single sample [64], [66]. Microfabricated electrode designs can add functionality as well, with the small dimensions possible opening new possibilities for detection. Interdigitated electrodes with sub-micron gaps have been used to detect DNA hybridization [67]. Using aptamers for recognition, Löhndorf *et al.* created microfabricated gold electrodes with nm spacing between them [64]. This impedance-based biosensor system detects the change in dielectric constant between the electrodes when aptamers bind to their target protein.

For some of the previously described detection techniques, such as mass-based cantilever systems, microfabrication is a necessity. The individual design, materials and microfabrication techniques can have a significant impact on the sensitivity and characteristics of microfabricated cantilevers [63].

As microfabrication techniques result in smaller, more sensitive active detection areas in biosensors as well as arrays biosensors in a single device mean that the exact delivery of biological samples to these components becomes more relevant. Additionally, the final goal of many biosensors is to make portable devices that can be operated outside of laboratory environments and are also easy to operate. Incorporating microfluidic systems into biosensor devices can address both of these concerns. Microfluidic components can accurately deliver biological samples and reagents to active areas of biosensors and are necessary for making complete portable devices such as with lab-on-chip technologies [68]. Aptamer-based biosensors have been created which take advantage of microfluidic channels for reagent transport [69] as well as systems which rely on microfluidic transport for detection [70].

Inoue *et al.* designed an aptamer array on a microfluidic chip for the on-site detection of thrombin in blood samples [69]. Detection for this device is based on a change in measured SPR based on thrombin binding to aptamers bound to the chip. The microfluidic aspect of this design allows for very simple sample loading, only requiring loading the sample port of the microfluidic chip and letting capillary action carry the sample over the aptamer treated sections of the chip, allowing for reliable on-site sample collection [69]. Measurements of recognition are done in a separate SPR reader, but this microfluidic integration is still an important step towards a fully integrated portable aptamer-based biosensor system.

Microfluidic components can be used in biosensor systems for more than just simple sample transport. A design proposed by Lin *et al.* uses microfluidic flow to wash away impurities and increase the concentration of a target biomolecule [70]. The principle of this system is, like the previously discussed design, have a sample solution flow over an area with bound aptamers. In this case, once target biomolecules (in this case AMP [70]) are bound to the aptamers, sample solution continues to flow, thus removing any extra impurities in the sample and collecting more the target biomolecule. Once it is concentrated sufficiently the area with attached aptamers is heated (using an on-chip heating element) causing the aptamers to denature and release the target biomolecules. These are then collected and analyzed [70].

B. CMOS System Integration

The majority of the aptamer-based biosensor designs discussed previously are still in early stages of development, and as a result finalized sensor hardware has yet to be designed. Increasingly more frequent research, however, is focusing on integrated CMOS (Complementary Metal-Oxide-Semiconductor) designs of compact and effective biosensor devices [71]. Using CMOS technology, very small and efficient biosensor circuits can be created and the sensor electrode (often gold) for the biosensor can be simply patterned onto these circuits using the same deposition techniques that were used to build the rest

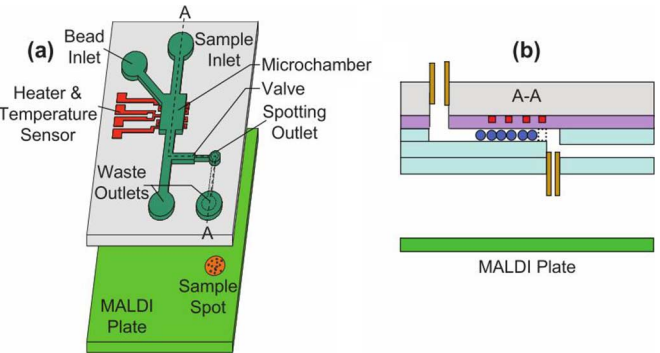


Fig. 11. Microfluidic aptamer-based biosensor device designed by Lin *et al.* [70]. The microchamber contains microbeads functionalized with aptamers for the detection of AMP. Sample solution flows from the inlet to the waste outlet until the target biomolecule is concentrated and impurities are removed. The chamber is then heated, releasing the target biomolecules and the valve is opened, allowing for collection and analysis. (a) A top down view of the device design. (b) A side view of the device along line A (reprinted with permission of IEEE).

of the sensor [71]. These designs are generally for electrical or electrochemical biosensors for the detection of proteins or DNA [72]–[75]. Although these designs have not been made specifically for aptamer-based sensors, they could easily be adapted for such applications (by using aptamers rather than antibodies or other such biological detection elements). There have already been other thorough reviews on the subject of CMOS biosensor design for a variety of detection and recognition techniques including excellent reviews by Thewes *et al.* [66], [71], [76], Jang *et al.* [77] and Wang *et al.* [78].

Manickam *et al.* created a CMOS electrochemical impedance spectroscopy (EIS) biosensor array for the detection of DNA and proteins [72]. These arrays are capable of detecting proteins and DNA with a high detection dynamic range in real time without labeling. Additionally, by designing multiple sensors on a single IC chip (in this case, a 10×10 array), multiplexing is possible on a single device. By integrating CMOS device circuitry directly with a gold attaching layer (and with proper electrical isolation, attached antibodies and DNA), these devices are promising as versatile, portable and cost effective EIS biosensors. A similar, 16 channel biosensor was created by Jafari *et al.* which utilizes frequency response analysis for DNA detection [73]. Other CMOS biosensors use voltammetry along with redox probes (in a very similar way to some of the electrochemical detection techniques mentioned above for aptamer biosensors), such as the sensors designed by Levine *et al.* [74].

Beyond basic electrical spectroscopy biosensors, other electrical CMOS biosensors have been designed. Capacitive biosensors which use large arrays of nano-electrodes for sensing (which consist of polished copper metal vias) have been made on small CMOS chips for purposes in multiplexing microfluidic biosensors [79].

Although there are many variations in their individual designs, CMOS biosensors, in general, offer compact, cost-effective methods for simultaneously detecting multiple analytes due to their highly integrated designs (combining all necessary detection circuitry directly with metal electrode layers) and large arrays of electrodes on single chips. Many of these designs have

TABLE I
SUMMARY OF DISCUSSED DETECTION TECHNIQUES AND GENERAL DETECTION LIMITS

Method	Description	Detection Limit
Fluorophore	Upon binding with the target biomolecule, the emission of fluorophores attached to aptamers changes.	1mM (ATP) [39]
Fluorophore + quencher	The distance between a fluorophore and quencher attached to an aptamer changes upon biomolecule binding.	0.3nM (thrombin) [40]
GNP colourmetric	The presence of the target biomolecule on aptamers attached to GNPs prevents GNP aggregation, causing a difference in optical density.	8nM (mucin 1 peptide) [7]
QD FRET	Target biomolecules displace labeled DNA segments, preventing FRET between quantum dots and the fluorophore labels.	0.024mM (ATP) [14]
Basic redox label electrochemical	Target biomolecule binding causes aptamers bound to an electrode to change conformation such that attached redox labels cause electron transfer.	0.06nM (interferon) [11]
Label-free electrochemical	Measures the change in interfacial electron impedance caused by protein attachment to aptamers bound to an electrode	0.1nM (thrombin) [31]
GNP enhanced redox label electrochemical	GNPs with attached aptamers and redox labels are brought in close proximity to a gold electrode through binding with target biomolecules.	100fM (thrombin) [57]
CNT transistor	The electrical properties of CNT transistors with attached aptamers change with the target biomolecule binds to the aptamer.	250pM (IgE) [13] Single-molecule detection (thrombin) [59]
QCM	The frequency of oscillation of a QCM changes due to the added mass of target biomolecules attaching to aptamers.	50pM IgE [9]
Cantilever	Cantilevers with attached aptamers deflect when target biomolecules attach.	50pM (TAQ DNA polymerase) [24]

been successful in detecting proteins and DNA and could potentially be easily adapted for aptamer-based detection.

V. APPLICATIONS AND COMPARISON TO OTHER METHODS

As mentioned previously and in some of the above listed detection method descriptions, aptamer-based biosensors have been designed for a wide range of applications. This diversity is due to the great variety of targets for which aptamers can be designed. The versatility of aptamers also means that many of the previously discussed sensor designs can work with many of these applications. In most cases, as long as a suitable aptamer can be made, multiple detection methods can be used for certain applications.

Aptamers which bind with Ochratoxin A, a mycotoxin which can be present in cereal products, have been used as the basis of several biosensor designs proposed for food testing [19], [21]. In addition to specific toxins, aptamers have also been created for the detection of proteins produced by bacteria which are common causes of food contamination [22]. The final goal for these sensors is to be used for other similar toxins as well, thus these sensors could test for a range of contaminants in food

stock before distribution and be adopted as a part of regular food screening.

Similar applications have been proposed for environmental screening. Aptamers can be created which can bind with a variety of small molecules of interest in environmental testing (contaminants, toxins, etc.) however there have not been as much research into making these types of sensors as ones for other applications [22].

Many of the previously stated examples of aptamer-based biosensors, as well as many others, are for use as tools for medical diagnoses. The designed target biomolecules for these sensors include proteins, metabolites and other small molecules. By measuring either individual concentrations of such molecules, or by measuring the levels of multiple target biomolecules at one time, specific biological conditions can be monitored, or diseases can be diagnosed.

Using aptamers for biomolecular recognition is only one of many techniques being used in biosensors and biosensor research. As with the previously discussed reporting techniques, each detection technique has certain strengths and weaknesses. Some techniques are better suited to certain biomolecules and

testing constraints such as required testing time, cost, detection limit and sample background. Unfortunately, the most accurate techniques are often impractical and too expensive for many biosensing applications [6], [58].

In terms of recognition elements, it is easiest to compare the relative merits of aptamers and antibodies. Although they are very similar, in that they both selectively bind to specific molecules, there are some key differences. These differences and their differing implementations are worth further discussion. In terms of production, aptamers are easy to prepare in large quantities (using PCR) with very little variation (less than in antibody production) [23], [47]. Also, unlike the standard procedures used in making antibodies, the SELEX process for making aptamers is a fully chemical process that does not involve the use of animals or bacteria (again leading to more consistent production). Aptamers are generally more stable than antibodies. In particular, they bond well to surfaces and are not easily or permanently damaged by temperature fluctuations [23]. At around 100 base pairs in length, most aptamers are smaller than antibodies [6], [47]. This fact can be exploited in biosensor designs by allowing a larger, denser concentration of recognition elements to adhere to surfaces, or for target biomolecules to be brought in closer proximity to sensor devices (such as in the CNT-FET sensor described above) [13]. Perhaps the most significant advantage of aptamers over antibodies lies in the versatility of aptamers; not only in the variety of possible targets, but also in the variety of possible applications. Antibodies are only suited for targeting immunogenic compounds such as proteins, whereas there is almost no limit to what aptamers can be designed to target [47]. The fact that some aptamers not only bind specifically with targets, but undergo predictable, consistent conformational changes when they do leads to a number of new possibilities.

Many of the above-mentioned detection methods rely on conformational changes to function and therefore can only work with these structure switching aptamers. Despite all of these advantages, there are still some important limitations preventing aptamers from being more widely utilized. Being a relatively new field of research, aptamers are generally not as well established as antibodies. Although aptamers can be made to bind to a wide variety of targets, there are still many aptamer-target pairs which have not yet been characterized or produced [15] and in some cases with smaller molecular targets, aptamers have a lower detection limit than other recognition elements [80]. Also, even though aptamers can be made which bind to a wide variety of targets, not all of these aptamers will have the desired structure switching properties. Therefore, just because an aptamer can bind to a specific target this does not mean that the aptamer can be used in all biosensor applications. This can be a serious limitation to the implementation of certain aptamer biosensor designs.

The following table briefly summarizes most of the biosensor designs discussed. It is important to note that although some of these techniques have clearly superior detection limits, this does not mean that they are superior in all aspects. Other, less sensitive biosensor designs may be less complex, less expensive and be suitable for different applications than more sensitive designs. The reported detection limits are based on one example

of specific research for specific targets only. They are meant to give a general idea of the detection limits for that type of detection design, not a finalized limit. The detection limits for different designed targets can vary as well as the type of sample tested.

VI. CONCLUSION

Aptamers offer many promising opportunities in applications in biosensor circuits and systems. Not only do they lend themselves well to biosensing, but also because of their stability, ease of production and high specificity, they have many unique properties that can be exploited in a number of novel detection techniques. Aptamers have been shown to be well suited for biosensors that utilize a variety of optical, electrochemical and mass based detection techniques. Even when used along with detection techniques compatible with other recognition elements, such as antibodies, aptamers have been shown to create biosensors that are superior in terms of sensitivity, stability and limit of detection. CMOS based biosensor designs have been studied for protein and DNA detection. These offer compact highly efficient multiplexing designs that could be easily adapted for use with aptamers as recognition elements. One of the main problems limiting aptamers from being used in more applications lies in the fact that not all aptamers are compatible with certain standard detection techniques. Further research is therefore required to identify or design aptamers that are better suited to not only a wide variety of targets, but also to a variety of detection methods. Future integration of aptamer-based detection with microfluidic systems and microfabrication techniques could overcome this and lead to portable, effective, versatile biosensors for numerous potential applications.

Incorporating microfluidic systems with aptamer-based biosensor designs would further simplify and miniaturize devices. Microfluidic channels could carry sample solutions from a large input port to the active area (or areas) of the sensor, eliminating the need for laboratory technicians to process samples. In-device electronics or other miniaturized devices could be used to detect target biomolecules (through electronic detection, electrochemical detection, optical detection etc.). The resulting biosensors could be operated directly by doctors in hospital or non-clinic settings for health monitoring, for on-site environmental testing or even by people to monitor their own health.

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