Chapter

Aptamers for Infectious Disease Diagnosis

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Abstract

Aptamers are in vitro-selected, nucleic acids with unique abilities to bind strongly and specifically to their selective targets (ligands) based on their three-dimensional structures. Target binding is generally associated with a change in aptamer structure, which provides a means of linking many output signals to the binding event. Being synthetic, aptamers are less expensive compared to antibodies. Aptamers are also more easily modified chemically or their sequence changed to optimize properties such target specificity, storability and stability. In this chapter we will discuss the potential benefits of applying aptamers to diagnostics with a focus on infectious disease and the unique challenges posed by aptamers for their successful incorporation into reliable aptasensors.

Keywords: aptamers, SELEX, aptasensors, portable diagnostic tools, electrochemical impedance spectroscopy

1. Introduction

Aptamers, first disclosed in 1990 by three groups [1–3], are ssDNA or RNA molecules capable of binding strongly and specifically to their target (ligand) molecules. Their target binding specificities and affinities are based on their sequence-specific 3D structures. Such properties of aptamers make them analogues of antibodies with unique advantages. For example, aptamers are relatively small (diam. ~2 nm) compared to antibodies (diam. ~15 nm), which allows them to bind targets that are inaccessible to the larger antibodies. Like antibodies, their properties are defined by the ionic conditions and pH in which they are placed. However, being shorter polymers, aptamers are generally more sensitive than antibodies to their physical and chemical environment.

In contrast to the time-consuming and expensive production and screening procedures for antibodies, aptamers can be produced faster and more cost effectively by a procedure known as Systemic Evolution of Ligands by Exponential Enrichment (SELEX). Once an aptamer sequence has been identified, its further production is by chemical synthesis, for which variation is negligible compared with the batch to batch variation of antibodies generated in animals or by cell culture. Their synthetic production makes aptamers accessible for selective chemical modifications to enhance their binding specificity or to increase their resistance to degradation. With such advantages over antibodies, aptamers have emerged as new generation molecular recognition elements [4]. In the current chapter, we focus on their impact in diagnosis of infectious disease agents. The reader is referred to other reviews of the application of aptamers to therapy, biosensing and molecular probing [5–10].

2. Aptamers in diagnostics

Fast and accurate diagnosis is a key factor for the treatment of infectious disease. Molecular recognition by aptamers can be highly discriminatory such that they can distinguish between two closely related molecules, including conformational isomers [11] or highly related proteins [12–14]. One-to-one comparison between aptamers and antibodies as recognition elements for the same target molecule, demonstrated that aptamers can equal antibodies in their sensitivity and selectivity [15]. As an added advantage, aptamers showed tolerance to repeated regeneration and recycling after ligand binding.

The small sizes and homogeneous structures of aptamers allow them to be immobilized in a dense and well-oriented manner. The higher density of aptamer packing compared with antibodies increases the binding capacity of the sensors and extends their linear range of detection of analyte [9, 15]. With these aspects of aptamers considered, their application as recognition elements in analytical devices offers a multitude of advantages and brings a new dimension to diagnostics.

Aptamers are compatible as the recognition element with many sensor platforms, including quartz crystal microbalances (QCM), surface plasmon resonance (SPR), diamond field effect transistors (FET), electrochemical impedance spectroscopy (EIS), colorimetric and fluorescence-based optical detection. Of these, electrochemical impedance spectroscopy (EIS) has gained popularity as it offers rapid, low-cost, label-free detection with high signal to noise ratios and sensitive detection of target molecules when employing aptamers [16, 17]. EIS is more sensitive than other electrochemical techniques [18] and is one of the best techniques to analyze the properties of electrochemical systems [19]. EIS is a technique used to study the electrochemical response to the application of periodic small amplitude ac signal at different frequencies [20, 21]. It is useful to monitor the changes in the electrochemical properties of the system due to biorecognition events at the surface of modified electrodes. For example, the electrodes can be modified with aptamers to detect the presence of a target analyte. EIS produces high quality data by directly converting a biological event into an electrical signal. Moreover, EIS-based sensors are small and portable and can be employed outside of well-equipped laboratory. EIS is an attractive technique for biosensor development as it provides the advantages of real time monitoring and label-free detection and is compatible with flexible electronics, disposable sensors and wearable devices. Inkjet printing can be applied to produce aptamer-based EIS biosensors for their automated mass production with uniform aptamer deposition [22–24].

In some instances aptamers can also be used for therapeutics or for combined diagnostics and therapeutics (theranostics). Examples include (1) various aptamers to human immunodeficiency virus (HIV) proteins that either prevent virus entry or replication [7, 25–29], (2) the S-PS$_{8.4}$ aptamer that recognizes *Salmonella enterica* and inhibits invasion of the bacteria into human monocytes [30], (3) two DNA aptamers A9 and B4 against the H9N2 avian influenza virus that inhibit viral infection [31], and (4) a DNA aptamer against MUC-1 that was applied in a nanocomposite for fluorescent imaging and demonstrated to inhibit the proliferation of colorectal (HT-29) and breast (MCF-7) cancer cells [32].

Although antibodies dominate the global market of diagnostics and therapeutics, several biotechnology companies have actively started exploring aptamers for diagnostics. The first to develop aptamer-based diagnostic arrays, SomaLogic employed SOMAmers (slow off rate modified aptamers) to detect many protein biomarkers for disease diagnosis [33, 34]. The combination of aptamers as the recognition elements, with long shelf lives at room temperature, inkjet printing

methods for immobilizing them and EIS as the method of detection will enable the development of low cost, label free, and rapid response diagnostic devices that could be in disposable or wearable forms as well as in more conventional instrument formats.

2.1 Aptamers that detect biomarkers of microbial infections

Early detection of infectious disease is of primary importance for its management. The conventional methods of diagnosis, which include microbiological methods (isolation, growth and microscopy of pathogens), polymerase chain reaction (PCR) and immunological methods [35, 36] suffer from turn-around times of 24 h or longer. This is especially a problem when the patient(s) are located in remote areas from which samples must be sent to centralized laboratories for their analysis. It is also difficult to grow some microbes in culture, which limits their detection.

Viral diseases are generally detected by serology, immunology or PCR amplification of DNA/RNA fragments corresponding to the pathogen's genome. Although it is sensitive and specific, the performance of PCR requires appropriate instruments, specialized reagents and experienced personnel. Immunological methods, which are widely used for diagnosis, employ antibodies specific to a protein or carbohydrate moiety that is unique to the target pathogen. Some popular immunological methods are the agglutination, ELISA and western blot assays. These well-established and time-tested assays are the work-horses of modern clinical laboratories. They are generally reliable and are likely to be the mainstay of the clinical technical repertoire well into the future. But, these assays limit the ability of communities to respond rapidly to microbial and viral outbreaks because they require laboratory equipment that is not readily portable and trained personnel to perform them. ELISA and western blots also depend on provided antibodies, which require cold storage to prevent their denaturation. Infectious disease outbreaks often start in regions that are distant from clinical laboratories. Therefore, the challenge for future diagnostics is to develop portable devices that require little expertise to perform. Current technology development is moving in this direction with portable PCR machines [37] and lateral flow immunology tests [38]. Whereas the former still requires trained personal to operate, the latter can often be readily used and interpreted by an untrained individual.

Diagnostics based on aptamers stand out as promising options for rapid, cost effective and specific detection of pathogens using devices that can be operated by minimally trained personnel. Many aptamers have been reported that recognize specific viruses and bacteria. Some were selected against recombinant proteins from the target microbe or virus. Others were selected against the intact microbe or virus. For example, RNA aptamer S-PS$_{8.4}$, which specifically recognizes the type IVB pili of *Salmonella enterica*, was isolated using a recombinant pilin structural protein as the selection target [39] and incorporated into a potentiometric biosensor as a recognition element for *S. enterica* [40]. This aptasensor could detect a single CFU of target *S. enterica* and was specific for *S. enterica*, not recognizing either *Escherichia coli* or *Lactobacillus casei*. Using a cell-SELEX approach, two 62 nt DNA aptamers, SA17 and SA61, were selected against intact *Staphylococcus aureus*. As for S-PS$_{8.4}$, these aptamers bound their *S. aureus* target with high affinities and specificities [41]. Many aptamers have been selected with specificities for particular microbe targets including or *Campylobacter jejuni* [42, 43], *L. monocytogenes* [44–48], *Vibrio parahemolyticus* [49], *Shigella dysenteriae* [50], *Streptococcus pyogenes* [51], *Francisella tularensis* [52], *Pseudomonas aeruginosa* [53] and the spores of anthrax *Bacillus anthracis* [54, 55]. Parasites are also good aptamer

targets with aptamers identified that recognize Trypanosoma spp., Plasmodium spp., Leishmania spp., *Entamoeba histolytica*, and *Cryptosporidium parvuum* [56]. For the diagnosis of invasive fungal infections, DNA aptamers have been screened against (1 → 3)-β-D-glucans from cell wall of *Candida albicans*. Two selected DNA aptamers (AU1 and AD1) showed high binding affinities in the range of 100 nM and did not bind to the same domain of (1 → 3)-β-D-glucans. The application of these aptamers in a double-aptamer sandwich enzyme-linked oligonucleotide assay (ELONA) resulted in an assay sensitivity and specificity of the detection of ~92% [57]. For viruses, there are aptamers that recognize HIV intracellular proteins [7, 13, 58–60], the HIV envelope glycoprotein [28, 61], hepatitis C [62–64], influenza virus [65–67], herpes simplex virus 1 [68], dengue virus [69], zika virus [70] and ebola virus [71]. In another study, a device was reported for the multiplexed detection of the envelope proteins of Zika and chikungunya viruses. The detection takes place in a microfluidic channel containing microsized pillars with attached aptamers. These pillars increase the surface sensing area, thereby enabling the attachment of more aptamers and increasing the overall sensitivity of the sensor envelope proteins. The working principle of this device depends on the formation of a protein-mediated sandwich with an aptamer-functionalized gold nanoparticle (AuNP) and an unattached aptamer. The signal is obtained upon introduction of silver reagents into the channel, which is selectively deposited on the AuNP surface, providing a gray contrast in the testing zone. This colorimetric aptasensor is reported to detect clinically relevant concentrations of Zika and chikungunya envelope proteins in phosphate-buffered saline (1 pM) and calf blood (100 pM) with high specificity [72].

Many of the aptamers discussed have been employed as recognition elements in diagnostic tools of a wide variety of types with electrochemical sensors being a popular platform. Examples include a potentiometric carbon-nanotube system to detect trypanosomes in blood [73], a voltametric aptasensor for ultrasensitive detection of *Mycobacterium tuberculosis* (MTB) virulence factor antigen ESAT-6 [74] and an EIS aptasensor for influenza virus [67].

Along with fast read-out, another advantage of the electrochemical approach is high sensitivity. The aptamer-based detection threshold is sometimes lower than for RT-PCR as demonstrated for influenza virus [67]. In another example, aptamer conjugates with gold nanoparticles were sensitive enough to detect a single *Staphylococcus aureus* cell [41]. An aptasensor has been reported that detects attomolar concentrations of the variable surface glycoprotein from African trypanosomes as analyte in blood [73]. From these and other examples it was found that immobilization of aptamers on biosensor surfaces increases target binding affinity [75–78]. The increased affinity is most likely due to two effects of immobilization: (1) immobilization creates a multivalent surface that decreases the rate at which the aptamer ligand can leave the surface (off-rate), which is the denominator in calculating the association constant ($K_a = k_{on}/k_{off}$), and (2) molecular crowding promotes aptamer folding to produce the appropriate ligand-binding structure [79–81].

Due to their low concentrations in the blood, infectious disease markers can be difficult to detect [82, 83]. Here, aptamers can play a different role of concentrating the target prior to their quantification. For this purpose, magnetic beads coated with aptamers specific for *Trypanosoma cruzi* were used to capture these parasites from the blood in which they are present at very low concentrations [84].

The stage is set for the development of commercial diagnostics for infectious disease agents. Some have already come to market such as OTA-Sense and Aflasense developed by Neoventures Biotechnology Inc. for detection of toxins in food samples, AptoCyto and AptoPrep developed by Aptsci Inc. for isolation of biomarker positive cells, SOMAscan from SomaLogic for diagnosis of several diseases, CibusDx a food pathogen Diagnostic platform developed by the

USA-based start-up (Pronucleotein, Inc.), OLIGOBIND®Thrombin activity assay by Sekisui Diagnostics [85]. Aptasensors demonstrate remarkably short detection times compared with the conventional methods of ELISA and PCR. The advantage of a faster detection time is of utmost importance for identifying rapidly developing and epidemiologically dangerous diseases, such as influenza, Ebola and SARS (Severe Acute Respiratory Syndrome). The rapid detection capabilities of aptasensors and their ready portability will broaden their scope of acceptance in the field of diagnosis.

3. Aptasensors

Some of the most attractive features of aptamer-based sensors (aptasensors) are their stability to storage at ambient temperatures and their reusability. Moreover, their small size and versatility allow aptamers to be immobilized at high densities, which facilitate their multiplexing in miniaturized systems. Several signaling modes have been coupled to aptamer-based sensors [86, 87]. Some popular outputs for detection include fluorescence [88], chemiluminescence [55], electrochemical [89], field effects (FET) [90], surface plasmon resonance (SPR) [91], changes in resonating frequency of quartz crystal sensor (QCM) [92], surface acoustic waves (SAW) [93], mechanical (microcantilevers) [94]. In this chapter, we will focus mainly on aptamer-based biosensors with fluorescent or electrochemical outputs.

3.1 Fluorescent aptasensors

Aptasensors with fluorescence outputs are designed to take advantage of the flexibility of aptamers, which results in their frequently adopting alternate conformations in the presence or absence of their target molecules. For these sensors, aptamers are modified in key positions with fluorescent dyes that interact in Förster resonance energy transfer (FRET). Upon aptamer binding to its target, the associated structural change alters the distance between the fluorescent dyes and thus the efficiency of energy transfer. The signal change, manifested as an increase (signal-on mode) or decrease (signal-off mode) in fluorescence, is proportional to the extent of target binding. A representative "signal-on" fluorescent aptamer holds a fluorophore, usually at one end, which is quenched by a molecule that is attached to a proximate location in the unoccupied aptamer. Target binding separates fluorophore from quencher allowing recovery of the fluorescent signal, which provides a quantitative measure of the target concentration [95]. FRET can also be used in "signal-off" sensor designs in which the conformational change of the aptamer on target binding brings the donor and quencher into closer proximity with a resulting fluorescence quenching. Sensors based on the "signal-off" mode are usually less sensitive than those based on the "signal-on" mode, but they can help to improve target detection by low-affinity aptamers [88].

An alternative means of signaling an aptamer binding event using fluorescence is with an oligonucleotide (attenuator) that is complementary to a portion of the aptamer and remains bound to the aptamer in the absence of target molecule. The length of the attenuator and its placement on the aptamer must create a condition that prevents aptamer folding to the actively binding conformation, but the affinity of the aptamer for the attenuator should be less than for the target molecule. With these requirements fulfilled, the target molecule can successfully compete with the attenuator to bind the aptamer and release the attenuator. Such a design can be used for "signal-on" reporting if the target and aptamer are labeled with fluorescent dyes that are optimally placed to interact in FRET while the aptamer and attenuator are

hybridized. This format can also be used for a "signal-off" system in which a single fluorophore is attached to the attenuator. When the aptamer binds the target molecule, the released attenuator adsorbs to surrounding gold nanoparticles (AuNPs), which quench the fluorescence [96]. Another aptasensor design used upconversion nanoparticles (UCNPs) as donors and AuNPs as acceptors for rapid, ultrasensitive and specific detection of bacteria (e.g., *E. coli* ATCC 8739) [97]. FRET-based aptasensors provide an efficient method for detecting pathogens and their released toxins in one step [98–100].

The concept of using a material to quench the fluorophore was applied to create a paper-based MoS_2 nanosheet-mediated FRET aptasensor for rapid malaria diagnosis [96]. This format uses paper test strips impregnated with fluorescently-labeled aptamers and MoS_2 nanosheets. The MoS_2 quenches the fluorescence until the aptamers are released when they bind their targets. These aptasensors are facile, inexpensive and therefore attractive for point-of-care diagnosis, especially in low-resource areas. Similar "low-tech" FRET-based aptasensors have also been found to be ideal for spacecraft, such as for diagnosing microgravity-induced bone loss in outer space by monitoring urinary C-telopeptide [4, 101]. In such scenarios, where both space and lab resources are limited, handheld fluorometers such as the commercially available QuantiFluor™ (Promega Corp.) or other such portable fluorometers will provide much needed opportunities for point-of-care diagnostics. These applications benefit from the greater stability to ambient temperatures for storage of aptamers compared with antibodies.

3.2 Electrochemical aptasensors

Upon binding to their target molecules, aptamers fold their supple, single-stranded chains into distinct three-dimensional (3D) structures. This structural change can be employed for initiating electron-transfer when the aptamers are labeled with a redox-active moiety and immobilized on a conducting support. Several electrochemical aptasensors have been developed based on this strategy [87], which can also be classified into "signal-on" and "signal-off" aptasensors. For example, an electrochemical thrombin aptasensor was constructed by immobilizing a thrombin aptamer (TBA) labeled with redox-active methylene blue (MB) on an electrode [102]. After binding thrombin, the TBA adopts a G-quadruplex structure, which moves MB away from the electrode. This "signal-off" sensing format has the disadvantage of a decreasing signal with increasing target molecule. An example of the preferred "signal-on" format includes the TBA, which is immobilized on a gold electrode and tagged with a terminal electroactive ferrocene redox label [103]. In the absence of thrombin a low signal is produced because many of the conformations adopted by the aptamer do not bring the ferrocene close to the electrode. Upon binding thrombin, the TBA adopts a G-quadruplex conformation, bringing the ferrocene to the electrode to allow electron-transfer and a positive signal in the presence of target molecule.

Electrochemical signals can be amplified when catalytic events are part of the signaling mechanism. For example, an electrochemical aptasensor was developed to rapidly diagnose tuberculosis (TB) by detecting the *Mycobacterium tuberculosis* antigen, MPT64, in serum samples [104]. MPT64 exists in serum as a disulfide linked homo-multimer. With multiple target sites on the same multimeric particle, MPT64 can be detected by a sandwich assay with the same aptamer on each side of the sandwich. In this study, coil-like fullerene (C60)-doped polyaniline (C60-PAn) nanohybrids were used as redox nanoprobes and catalysts to initiate the oxidation of ascorbic acid. When linked to the MPT64 aptamer, these nanohybrids were brought close to a gold surface (also decorated with MPT64 aptamers) in a

sandwich joined by MPT64 multimers. In this configuration the electrons released by the oxidation of ascorbic acid were transferred to the gold electrode. This simple yet elegant approach for TB diagnosis showed selectivity to target antigen over several other serum proteins, a wide linear range of detection from 0.02 to 1000 pg/mL and a detection limit of 20 fg/mL MPT64. The delayed diagnosis and misdiagnosis of patients with MTB infection is the leading cause behind the spread and high mortality rate of TB [105]. Therefore, the possibility of rapid and accurate detection of MTB by these aptasensors is of great significance for the early diagnosis and treatment of TB.

Electrochemical impedance spectroscopy (EIS), an electrochemical label-free detection method, can be an extremely sensitive method for target recognition at the electrode/electrolyte interface [106]. Here, aptamers are immobilized on a gold (Au) electrode and the remaining gold surface filled in by a self-assembled monolayer such as mercaptohexanol (6-MCH). This approach was used with a DNA aptamer as molecular recognition element for malaria detection, for which the response range of 1 pM–10 nM covered the diagnostically relevant concentration range of Plasmodium lactate dehydrogenase protein from the falciparum parasite species (PfLDH) [107, 108]. The aptasensor functioned well with a sample matrix of 10% human serum and could be regenerated for reuse by washing with 6 M urea.

Electrochemical aptasensors have been fabricated to be sufficiently small to insert into a vein for continuous, real-time measurement of specific molecular targets in situ in the living body. The limited surface area of these small devices leads to low faradaic currents and poor signal-to-noise ratios when deployed in the complex, fluctuating environments found in vivo. To circumvent this problem, an electrochemical roughening approach was developed to enhance the signal-to-noise ratios by increasing the microscopic surface area of gold electrodes, thereby allowing more redox reporter-modified aptamers to be packed onto the surface. These high surface area electrochemical aptasensors of less than 200 μm in diameter were used in a proof-of concept study to measure continuous drug pharmacokinetic profiles over a 3 h period in live rats [109].

Colorimetric detection is gaining popularity in the diagnostic field considering its low cost and the minimal training needed to identify and interpret the visible signal. A colorimetric approach was used to develop a diagnostic device for tuberculosis with aptamers that bind to antibodies against the MPT64 protein secreted by *Mycobacterium tuberculosis*. When adsorbed to Fe_3O_4 magnetic nanoparticles (MNPs) the aptamers decrease the ability of the particles to reduce oxygen to H_2O_2 [110]. Upon exposure of the MNP-aptamer suspension to anti-MPT64 antibodies, the aptamers preferentially bind to the antibodies, thereby increasing the available surface area of the MNPs with the resulting higher rates of H_2O_2 production. Inclusion of 2,2′-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS), which is oxidized by H_2O_2 and converted to a colored product, signals the presence of anti-MPT64 antibodies [111]. Another format for a colorimetric aptasensor is a paper-based microfluidic chip. For this format, aptamers against bacteria considered as nosocomial and antibiotic-resistant were immobilized by ultraviolet crosslinking on a nitrocellulose membrane housed within the chip. Incubation with bacteria, washing and then the addition of biotinylated aptamers allowed the use of HRP-linked streptavidin to create a blue color based on the oxidation of tetramethyl benzidine (TMB) by the H_2O_2 product of HRP, which was trapped on the surface by way of its linkage to the streptavidin bound to the biotinylated aptamers attached to the target bacteria. This dual-aptamer microfluidic chip possesses many advantages such as rapid output (35 min), small size, higher specificity, and the capability to detect multiple pathogens simultaneously, which are ideal for point-of-care bacterial diagnostics [112]. A similar sandwich type aptasensor has been reported for the

early diagnosis of periodontitis, in which chronic inflammation is caused by many factors including pathogenic bacteria. Periodontitis is one of the major causes of tooth loss in adults. Aptamers were targeted against a potential biomarker of this disease, odontogenic ameloblast-associated protein (ODAM). The lateral flow strip format used a cognate pair of aptamers that recognize different sites on ODAM. One aptamer (2^0 aptamer) was attached to gold nanoparticles that were mixed with the sample to capture the biomarker. The second aptamer (1^0 aptamer) was present in a line on the strip to capture nanoparticles with attached biomarker. A control line with DNA complementary to the 2^0 aptamer captured the particles that did not have attached biomarkers. The biosensor had a detection limit of 0.24 and 1.63 nM in buffer and saliva samples, respectively [113].

4. Considerations for further development of aptasensor applications in diagnostics

Of the many aptamers that have been selected, very few have been applied as recognition elements in sensors and fewer have reached the stage of commercial availability. In this section we will consider some of the reasons that aptasensors, with all their promise, have been slow to come to the market in diagnostic devices.

One reason for the limited breadth of application of aptamers to diagnostics is that many of the biosensor platforms are new to the concept of incorporating aptamers as sensors. Therefore, the research focus has been on developing sensor platforms that are compatible with aptamers. For proof-of-principle devices, aptamers that have been previously demonstrated to function well on a variety of sensor platforms have been chosen as recognition elements. Consequently, the TBA and ATP aptamers have been incorporated into many sensor platforms [114, 115]. However, these targets are not relevant biomarkers for disease. With many sensor platforms now validated using these "prototype" aptamers the field has the opportunity to move forward to incorporate and optimize some of the many available clinically relevant aptamers for diagnostic applications.

Nucleic acids are more flexible polymers than polypeptides. Whereas there are two rotatable bonds between each amino acid side-chain in a polypeptide, there are six rotatable bonds between each base in a nucleic acid. This additional flexibility gives aptamers the property of ready structural rearrangement with target binding and enables their incorporation into many sensor platforms that rely on these rearrangements for creating signals. Thus, the fundamental principles underpinning antibody and aptamer-based sensors are different. Whereas antibody-based sensors rely on the uniformity of antibody structure and their bivalency, aptamer-based sensors rely on the flexibility of the monovalent aptamer structure and the structural changes that occur on target binding. Consequently, a single sensor format can be readily adapted to many antibodies, but each aptamer-sensor combination must be optimized to benefit from the unique structural change of the relevant aptamer. Thus, although there may be some applications for sensors that use the TBA and ATP model aptamers [116], their repeated use in developing and testing aptamer-based sensors has delayed the development of aptasensors for relevant biomarkers.

With the importance of aptamer structure and conformational changes for sensor development, a second challenge for reliable aptasensor development lies in the dearth of known aptamer tertiary structures. This deficiency results from several circumstances. First, biophysical determination of nucleic acid structure by conventional methods such as NMR and X-ray crystallography is more challenging than for proteins. Second, when structures can be determined by biophysical

means, these are often only the structures of the target-bound aptamers because the apo-aptamer structures are too flexible to be reduced to a single structure. Third, new aptamers are being reported at an increasingly rapid rate that is much faster than their structures can be determined. Finally, small modifications of existing aptamers that can have large effects on aptamer structure are often made for their application in sensors. Thus, aptasensors must be developed with little information about the tertiary structure of the aptamer employed and how it changes with target binding. The most likely route to obtaining molecular structure for most aptamers will be in silico modeling. Although not yet demonstrated to be adequate for accurately predicting the structures of short nucleic acids and how they change with target binding, molecular modeling techniques are improving and their successful integration into models for sensor mechanisms could eventually result in dependable strategies for engineering new aptamers and integrating them into sensors.

Many aptamer selection protocols require the availability of purified target molecules [117]. Protein target molecules are usually expressed as recombinant proteins by prokaryotic or eukaryotic cell cultures and then purified, frequently by affinity chromatography based on a capture tag. Like for obtaining antibodies, the protein targets must be pure. Difficulties can come if the recombinant protein is not post-translationally modified similarly to the native protein. For example, many biomarkers found in the blood are glycosylated in the native form, but the recombinant proteins produced by bacterial cells are not glycosylated. Due to their steric hindrance or by their altering the protein structure in the region of the aptamer epitope these modifications can make regions of a native eukaryotic protein inaccessible to aptamers generated against the recombinant protein equivalent expressed in prokaryotic cells [118]. An early screen for aptamers selected to bind non-glycosylated recombinant proteins should be to determine if they bind the native glycosylated protein. Approaches to selecting for aptamers that recognize glycan structure in the context of the protein will also be useful [119].

Cell-SELEX avoids the complication of the target lacking the native posttranslational modifications by selecting against the cell surface protein target in situ [120, 121]. However, the identities of targets obtained by Cell-SELEX are often not known. As well, Cell-SELEX is performed with cell lines that are different from normal or in situ cancer cells and that are cultured under conditions that differ from those in the body. In particular, cultured cells have adjusted to exist in serum, which is not present in vivo and to an environment of much higher oxygen content than cells in situ, both of which conditions might result in altered protein expression on the cell surface.

Another consideration for aptasensors is that they frequently must function with the target (analyte) in a complex sample matrix. Many sensors have been demonstrated to function well in simple buffers, while the most common biomedical samples (blood, serum, urine and saliva) are complex with many potentially interfering substances. Aptamer target binding affinity can be sensitive to the matrix in the range of dilutions commonly used to detect the analyte, such as 10 or 50% serum or urine [122, 123]. For biofluids, sampling methods must also be considered with a view to minimal invasiveness and small sample volumes.

As aptasensors are developed that avoid the pitfalls discussed here, these sensors will take their place beside the antibody-based assays and provide new capabilities such as continuous analyte monitoring and inexpensive devices that can be distributed to small clinics throughout the world and yet be connected by Bluetooth and other options to send their results to central clinics and distant physicians for improved monitoring of patients in rural and other isolated locations.

5. Prospects of aptasensors in next-generation diagnosis

Medical diagnostics is moving towards a future of individualized patient care. Individuals vary greatly in their response to specific drugs and in the rate at which these drugs are removed from the system [124–128]. Pharmacokinetic parameters have been reported to vary daily in the same patient [129] or with time of drug exposure [130] and between individuals depending on disease state [131–133], age [134], genetics [130, 135–139], concurrent medication [137], body fat composition [140], and even circadian rhythm [141]. Adherence to a drug intake regime is also a factor, particularly for care of the young and the aged [142–144]. For these many reasons, individualized diagnostics are considered a clinical necessity for improved patient treatment and for establishing effective therapeutic windows [145].

With the push towards individualized medicine as a desirable future approach for optimal patient care comes the need to move some diagnostics out of the clinic into the home. For this purpose, reliable inexpensive sensors that might be linked by wireless connections to clinical centers would be optimal. Some personalized diagnostics has long been available in the home. These include pregnancy tests and glucose monitors, which are based on antibody and enzymatic reactions. However, these are not linked to the larger medical care network. The course is now set for a huge expansion of personalized diagnostics that do not require trained operators on site, but that can transmit information to clinical specialists who can monitor a patient's condition off-site and continuously. This is a niche for which aptasensors can provide a large diversity of options with their potential for long shelf-live under ambient conditions, simplicity of operation, ability to be designed for continuous use or repeated use, and compatibility with wearable sensor formats.

Aptasensors can also be applied to address the acute need for diagnostics during infectious disease epidemics by their placement in clinics located in isolated regions of the world and in individual physician's offices that are distant from major well-equipped hospitals and clinical centers. With the additional capability of Bluetooth communication, centers of disease control can be quickly updated regarding the spread of infections, which will enable central authorities to rapidly initiate effective means of controlling a potential epidemic.

A future of diagnostics for individualized medicine and for the control of infectious disease outbreaks over vast regions will result from many cross-disciplinary collaborations that are already underway, which include experts in molecular biology, virology, medicine, engineering, diagnostics and other disciplines. With this effort, many of the aptasensors that are still now at the proof-of concept stage, are expected to become major contributors to a future of improved personalized health care for all people, including those living in remote regions, and will help to stem future outbreaks of infectious diseases.

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