Aptasensors Design Considerations

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Abstract. With the advancement of biotechnology, aptamer-based sensors have received intense attention in both research and commercial worlds. This is driven by the advantages of small molecule size, chemical stability, and cost effectiveness of aptamers over the conventional analyte detection using antibodies. This paper explores the aptasensors from a designer perspective and discusses the aptasensor design considerations by giving an overview of surface functionalization techniques and the existing mechanisms used to detect biomolecular interactions. It also expounds the factors that influence the accuracy and sensitivity of aptasensors

Keywords: aptamer, immobilization, aptasensor.

1 Introduction

Aptamers are synthetic nucleic acid isolated from large combinatorial libraries of oligonucleotides via an iterative in-vitro selection and amplification. This process is known as systematic evolution of ligands by exponential enrichment (SELEX). Aptamers are gaining popularity among molecular biologists due to their inherent advantage of superior specificity in binding a wide variety of protein and non-protein targets. Aptamers are smaller in size and have better physicochemical stability compared to antibodies. Furthermore, they are less prone to denaturation and can be easily modified to facilitate surface functionalization with long storage life. These advantages qualify them as an excellent biorecognition element in biosensors. A biosensor comprises of two main parts: a biorecognition element and a transducer that translates biorecognition event into measurable electrical, optical, or mechanical signals. Biosensors that employ aptamer as their biorecognition element are known as aptasensors.

To date, there exist a few excellent review papers [1, 2] that discuss aptasensors from different perspective. In this paper, the common surface immobilization techniques as well as the existing methods used to convert the aptamer-binding event into measurable signals are described. Factors that influence the analytical performance of an aptasensor are also addressed.

2 Surface Modification

Modification of the biosensor chip surface is a crucial first step that determines the specificity of a biosensor in detecting any analyte of interest. There are various

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immobilization techniques available, but the self-assembly technique is the most commonly employed method by many researchers. Self-assembled monolayer (SAM) involves dipping the substrate into a solution to allow adsorption of molecules on the surface. Physisorption involves non-covalent interactions between molecules such as electrostatic interactions, van der Walls forces and hydrophobic interactions. Surface immobilization on gold is usually performed via chemisorptions of three-segment thiol-terminated aptamers [3, 4]. The first segment, thiolalkane hydrocarbon, promotes formation of a monolayer on surface. Aptamer is extended from the monolayer surface via the second segment, a linker, to enable easy accessibility of aptamer binding site to target. The third element is the aptamer molecular probe that recognizes the target protein. To ensure proper orientation of surface immobilization onto thiol-functionalized silicon oxynitride waveguide, Johnson et al [5] used a cysteine-terminated STM scaffold protein.

Besides thiol, other functional group such as amine, biotin or carboxyl can be used to modify the terminus of an aptamer. Amine-terminated thrombin aptamers is implemented by Lee et al [6] to functionalize pyrolyzed carbon working electrode. Phosphate buffer saline (PBS) with Triton X-100 is used to remove unreacted functional groups. Non-specific binding of thrombin on the surface is prevented by blocking with 1% bovine serum albumin. Meanwhile, Bini et al [7] employed the layer-by-layer (LBL) approach. LBL is another form of self-assembly technique whereby multi-layers of biomolecules with opposite charged groups bond uniformly on the chemically modified surface. C-reactive protein (CRP) aptamer is immobilized via N-hydroxysuccinimide (NHS)-mediated chemical reaction and specific interaction between biotin and avidin.

3 Transduction Approach

Integration of a transducer in aptasensor is essential to convert the biorecognition event into measurable signals. This section will provide an overview of various transduction methods including reporters that assist the detection of aptamer-target binding event.

3.1 Electrochemical

Aptasensors that implements electrochemical detection consist of three electrodes: working, auxiliary, and reference. Detection of analyte of interest is achieved by measuring current or voltage changes generated by aptamer-target interactions at the electrode. Reporters such as catalytic labels, inorganic or organic catalysts, and nanoparticles are integrated in this type of detection system.

Commonly used electroactive labels are ferricyanide $[Fe(CN)_6]^{4-/3-}$ [8-10], ferrocene [11, 12], methylene blue (MB) [13, 14], and bis-anthraquinone-modified propanediol [15]. This type of detection technique exploits the conformational change of aptamer structure upon binding to its target. As shown in Fig. 1(a), in the signal "on" architecture, the electroactive end is distant from the electrode surface, limiting the electron transfer. Electron transfer increases upon target binding due to the conformational change of aptamer bringing the label to the surface. On the other hand, the

signal "off" architecture (Fig. 1(b)) yields a lower electron transfer upon target binding as the addition of a target disassociates the initially attached redox-tag.

In the work presented by Li et al. [8], small molecule such as adenosine is detected using part of the complementary aptamer strand in electrochemical impedance spectroscopy. This approach eliminates the dependence on conformational change of aptamers. Negatively charged adenosine-binding aptamer attached on the surface repels the negatively charged probe ($[Fe(CN)_6]^{4-/3-}$ anions. This causes high impedance that hinders the interfacial electron-transfer kinetics of redox probes. Impedance is reduced upon adenosine binding by releasing its negatively charged aptamer-target strand. The sensitivity of electrochemical detection can be amplified by incorporating polymerase chain reaction [16].

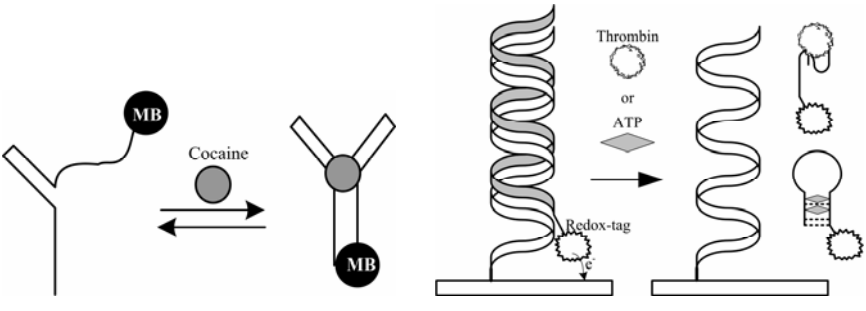

Fig. 1. Schematic representation of electrochemical detection based on (a) signal "on" (Adapted from [13]), and (b) signal "off" (Adapted from [15]) architectures

3.2 Electrical

Ion-sensitive field effect transistors (ISFETs) initially used for measuring pH has extended their usage in biomolecular interactions detection. This is due to their inherent advantage of robustness, small size, and cost effectiveness. Electrical sensing using field-effect transistor is a label-free approach. In this system, a reference electrode is exposed to the analyte solution. The source and drain deposited on silicon layer is separated by a thin insulating layer. The interfacial electron transfer resistance is controlled by the formation of target-aptamer complex. Hence, target molecules are identified by measuring the gate potential variations. Fig. 2 illustrates an example of ISFET-based aptasensor employed in adenosine detection [17].

Instead of using external reference electrode in ISFET-based transducer , Cid and co-workers [18] employed a network of single-walled carbon nanotube (CNT) as the transduction layer. The use of CNT in FET fabrication is favorable because it exhibits superior performance in terms of transconductance and subthreshold slope. An aptasensor based on aptamer-modified CNT-FETs for immunoglobulin E (IgE) detection has been developed by Maehashi et al [19]. Linker molecules are used to covalently bind 5′-amino-modified IgE aptamers to CNT. Alternately, So et al [20] modified the side wall of CNT with carbodiimidazole (CDI)-Tween for thrombin detection.

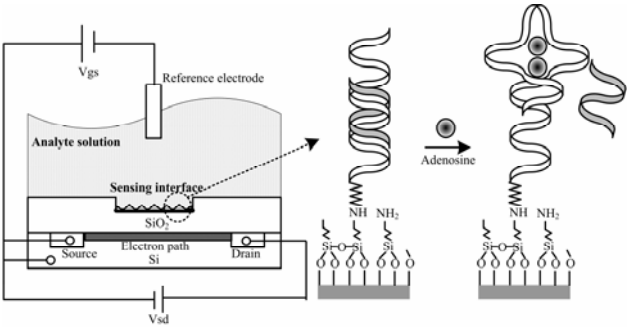

Fig. 2. Schematic diagram of an ISFET-based sensor (Adapted from [17])

Hydrophobic interactions bring Tween to the side walls while 3′-amine group of the thrombin aptamer is covalently bound to CDI moiety. The formation of aptamer-target complex shields the negative charges of aptamer, producing an increased height of Schottky barrier between the metal electrodes and CNT channel and thus leading to a decrease in the electrical response.

3.3 Optical

The most popular optical transduction method is the fluorescence detection. Fluorophore-quencher label pair is incorporated into DNA aptamer to detect the presence of L-argininamide in the work presented by Ozaki et al [21]. The structure of the DNA aptamer employed in this work is of a random shape without the addition of a target. As illustrated in Fig. 3 (a), when L-argininamide is injected, the binding of the target to the aptamer stabilizes the stem loop structure and subsequently quenches the fluorescence. Quantum dots have been implemented as an alternative to the fluorophore-quencher due to their robustness, stability, and the ability to tune via size variations [22]. Other optical transduction methods include the surface enhanced Raman scattering (SERS) [23] and colorimetric detection by Wang et al [24]. In the SERS method

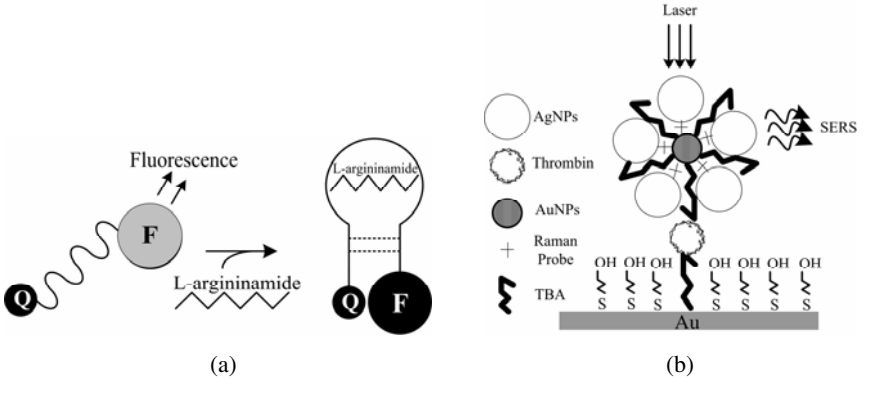

Fig. 3. Schematic representation of: (a) Fluorescent-quencher pair (Adapted from [21]), and (b) SERS transduction method (Adapted from [23])

(see Fig. 3(b)), thrombin-aptamer binds to thrombin at one binding site while gold nanoparticles (AuNPs) labeled with thrombin aptamer and Raman reporters are bound to the other binding site of thrombin target. Electromagnetic hot spots are generated on the AuNPs surface when target binds to aptamer. Raman signals are amplified via deposition of silver nanoparticles (AgNPs) on AuNPs surface. On the other hand, colorimetric detection implements dot-blot assay using aptamer-AuNPs conjugates whereby the AuNPs change color from colorless to red upon aptamer-target complex formation. The limit of detection can be further enhanced by using silver enhancement solution.

3.4 Mass

Formation of aptamer-target complex on the sensor surface generates changes in mass on the surface. Acoustics-based sensors measure the changes in mass for the target detection. This type of transduction method is also another form of label-free approaches. They operate based on piezoelectric effect and are effective in determining protein affinity on functionalized surfaces. An example of acoustic aptasensor is the quartz crystal microbalance (QCM) devised by Yao et al [25] to detect IgE. An electrical potential difference is generated between deformed piezoelectric surfaces when a pressure is exerted on a small piece of quartz. A detection limit of 2.5μg/l IgE in 5 min is achieved. To improve sensor's sensitivity, Lee et al [26] increased the amount of analyte molecules bound to the surface by implementing zinc oxide nanorod-grown QCM.

Surface acoustic wave (SAW) is another type of acoustic-based sensors. Interdigital transducers are employed to produce and detect acoustic waves at the guiding layer at the surface of the substrate. Detection of biomolecular binding is performed by measuring the frequency or phase change that corresponds to the mass on the surface. SAW love-wave sensor implements shear horizontal waves guided in a layer on the sensor surface to reduce energy dissipation of the acoustic wave in order to increase surface sensitivity. This detection method is employed in the detection of thrombin binding [27].

Surface plasmon resonance (SPR) spectroscopy [7, 28-30] is a surface-sensitive label-free technique. This method measures the variations in reflective angle upon adsorption of molecules on the metal surface. Information on the affinity and kinematics of biomolecular interaction is obtainable using this technique. Wang et al [31]

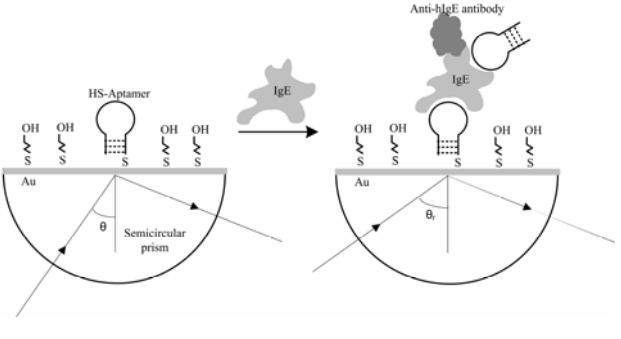

Fig. 4. Schematic of SPR detection. (Adapted from [31]).

implemented curvette-based SPR to characterize the interaction between IgE and aptamer (see Fig. 4) . Streptavidin and anti-IgE antibody are introduced during sensing to amplify the SPR signals. Eight-channel SPR sensor based on spectral modulation and wavelength division multiplexing is developed by Ostatná et al [29]. These sensing channels are formed by combining the wavelength division multiplexing of pairs of sensing channels with four parallel light beams.

Microcantilever provides a simple and label-free sensing mechanism. When molecules adsorb on to the surface, a difference in surface stress between the upper and lower surfaces causes the cantilever to bend [32, 33]. In static microcantilever sensing, this deflection is measured and it can be either positive (bend upwards) or negative (bend downwards) depending on the interaction between molecules [34]. As illustrated in Fig. 5, attraction between molecules will produce a positive deflection while repulsion interaction between molecules generates a negative deflection. Shu et al [35] demonstrated a V-shaped microcantilever detection using optical method to measure the deflection. This has been employed for the detection of human CDK2 in yeast cell lysate.

Dynamic microcantilever detects the presence of target protein by measuring frequency variations in an oscillating beam. Lu et al [36] incorporated a PZT actuator in their microcantilever. Sensitivity of the sensor is improved by separating the PZT actuator from the resonant structure to suppress energy dissipation. Q factor tends to decrease when cantilevers operate in liquid environment. To overcome this limitation, Li et al [37] introduced a new active magnetostrictive cantilever actuated by a time-varying magnetic field which exhibits a high Q value in liquid environment.

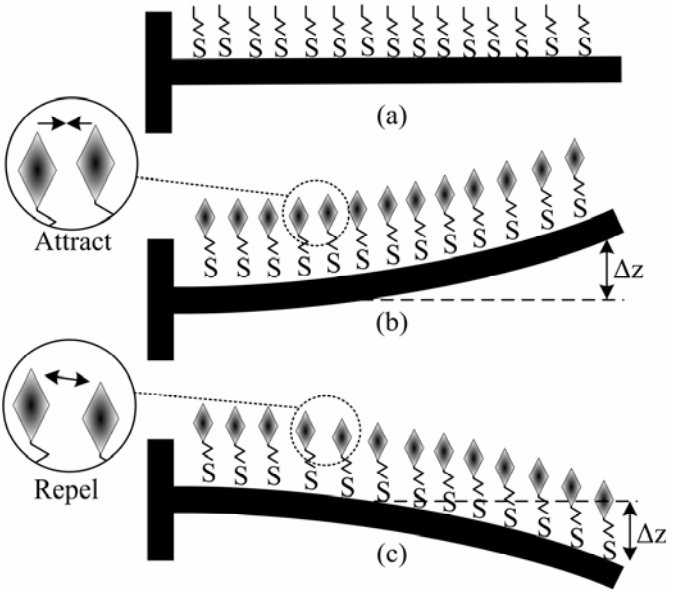

Fig. 5. (a) Before immobilization of target. (b) Attraction interaction between molecules causes the upward bending of cantilever. (c) Repulsion between molecules causes the downward bending of cantilever.

4 Sensor Performance

The immobilization procedure will determine the orientation of aptamers on the surface. These procedures directly affect selectivity and binding affinity of aptamers. The surface immobilization method is dependent on the functional groups (thiol, amine, or biotin) linked to the aptamers. Immobilization procedure that utilizes a linker such as avidin-biotin technique produces sensor with a higher sensitivity [38]. With the presence of linker, the binding site of aptamer is easily accessible to the target. The disassociation constant can be improved by integrating a spacer in between the binding moiety and aptamer. Liss et al. [39] achieved an improvement of disassociation constant from 8.4 nM to 3.6 nM. Regeneration of sensor surface is possible in aptamer-based biosensors because aptamers are less susceptible to denaturation compared to its antibody counterpart. This is a valuable characteristic as it allows the aptamer biosensor to be reused by removing target analytes using HCl without affecting its binding efficiency [12].

The structure of aptamer binding site is affected by pH. Effect of pH sensing solution on aptamer binding affinity was investigated using thrombin as the system model in [38] and [40]. The pH ranging from 4.7 to 8.5 has been tested. Denaturation of some biocomponents under extreme pH generates lower signal and higher background. The optimum pH for the thrombin model is found to be at pH 7.5. Binding affinity of aptamer is also influenced by ionic strength in the solution. Under non-optimal conditions, the presence of K^+ facilitates the maintenance of structural integrity in thrombin aptasensor [40]. Increased concentration of Na^+ in solution shields the negative charge on DNA aptamer leads to competitive binding between Na^+ and methylene blue to the aptamer. In addition, higher Na^+ concentration also contributed to the lower affinity of the aptamer. Meanwhile, aptamer-CRP complex presented in [7] exhibits a lower disassociation constant with the presence of 2 mM Ca^{2+} in the buffer. This is because Ca^{2+} provides higher stability to the aptamer-target complex.

5 Summary and Perspective

For effective detection of the binding of biomolecules, aptasensor needs to have excellent selectivity, accuracy, as well as stability for practical use. Comprehensive investigation of factors influencing the sensor performance is utmost important in achieving these goals. Immobilization procedure plays a major role in optimizing sensor sensitivity and specificity. Therefore, the selection of the immobilization method and type of aptamer linker and spacer to be used needs to be carried out with care and take surface regeneration into considerations. Optimal working condition is another factor that requires investigation in order to produce maximum sensor response. It is envisioned that the demand for high-throughput analysis and cost-effective biosensors will drive the development of label-free aptasensors since they circumvent the need to understand the mechanism of the binding and the labeling of the aptamer. With the advancements in biotechnology and bioengineering, it is anticipated that in future, aptasensors will be an integral part of lab-on-a-chip that will make instantaneous output results for quick decision-making. This will significantly advance point-of-care diagnosis.

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