Effects of diamond-FET-based RNA aptamer sensing for detection of real sample of HIV-1 Tat protein

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Diamond is a promising material for merging solid-state and biological systems owing to its chemical stability, low background current, wide potential window and biocompatibility. The effects of surface charge density on human immunodeficiency virus type 1 Trans-activator transcription (HIV-1 Tat) protein binding have been investigated on a diamond field-effect transistor (FET) using ribonucleic acid (RNA) aptamers as a sensing element on a solid surface. A change in the gate potential of 91.6 mV was observed, whereby a shift in the negative direction was observed at a source-drain current of \(-8 \mu A\) in the presence of HIV-1 Tat protein bound to the RNA aptamers. Moreover, the reversible change in gate potential caused by the binding and regeneration cycles was very stable throughout cyclical detections. The stable immobilization is achieved via RNA aptamers covalently bonded to the carboxyl-terminated terephthalic acids on amine sites, thereby increasing the sensitivity of the HIV-1 Tat protein sensor. The reliable use of a real sample of HIV-1 Tat protein by an aptamer-FET was demonstrated for the first time, which showed the potential of diamond biointerfaces in clinical biosensor applications.

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1. Introduction

Diamond is a promising material for merging solid-state electronics and bioelectronics that can be used with DNA molecules (Yang et al., 2002), proteins (Song et al., 2004; Hartl et al., 2004) and cells (Rezek et al., 2009; Dankerl et al., 2009). In recent years, many applications of biosensing using diamond as a material and electronically active devices have been developed. These include a field-effect capacitive electrolyte–diamond–insulator–semiconductor structure for the detection of penicillin (Abouzar et al., 2008), a capacitive sensor (Abouzar et al., 2009; Ingebrandt et al., 2011) and in-plane interdigitated electrodes that exhibit high sensitivity to molecules such as phosgene (Davydova et al., 2010). Capacitive sensors are generally simple to fabricate and operate and are widely applicable. However, a major drawback of capacitive sensing appears when it comes to miniaturization owing to the significant decrease in capacitance for small dimensions, causing sensing to become problematic. For miniature sensors, the electrolyte solution-gate field-effect transistor (SGFET) design is more suitable as it can provide a higher signal-to-noise ratio and good signal stability, its sensitivity can be increased and it can directly amplify signals (Kawarada et al., 2001; Kuga et al., 2008). The advantages of diamond SGFETs are that they are chemically resistant, biocompatible, can form a stable interface with biomolecules including covalent links (Song et al., 2006; Ruslinda et al., 2012) and can operate without gate oxides (Ruslinda et al., 2010). Hence, an increase in both sensitivity and selectivity is expected, resulting in new biosensing possibilities. Moreover, diamond does not require doping by impurities for device formation owing to the inherent feature of H-termination, which facilitates p-type surface conductivity when forming an interface with electrolytes (Ri et al., 1995; Maier et al., 2000; Sasaki and Kawarada, 2010).

The recent development of aptamers has led to increased interest in biosensor applications. Aptamers are single-stranded DNA or RNA molecules that have been selected from synthetic nucleic acid libraries for molecular recognition (Ellington and Szostak, 1990; Tuerk and Gold, 1990). The advantages of aptamers such as their simple synthesis, easy labeling, high stability and wide applicability have made them ideal recognition probes for protein or small-molecule detection. From the viewpoint of FET technology, if we control the pH and the concentration of buffer solution, aptamers are a superior choice because they are smaller (5–6 nm) than the Debye length (approximately 9 nm), thus providing binding inside a measurable range (Guo et al., 2005; Maehashi et al., 2007). As a result, binding between aptamers and target proteins can occur within the electrical double layer in a...
buffer solution, and therefore, an FET can easily detect changes in the charge distribution in the proximity of the diamond surface. Moreover, the density of immobilized aptamers on diamond channels can be controlled, and a high density of aptamers can easily be prepared. Therefore, these characteristics make aptamers ideal molecular recognition elements in analytical applications (Scheller et al., 2001) and versatile tools for therapeutics (White et al., 2000) and diagnostic applications (Brody and Gold, 2000). Yamamoto et al. were the first to develop an aptamer that yielded an efficient binding specificity against Tat protein but not against other cellular factors (Yamamoto et al., 2000). This RNA aptamer exhibits a binding affinity over 100-times higher than that of TAR RNA (59-mer) (Minunni et al., 2004; Tombelli et al., 2005). The aptamer has a TAR-like motif in its randomized region, with two adjacent bulging units with opposite orientations. The core element for binding to Tat is a four central pair helix flanked by two residues on each side (Yamamoto et al., 2000).

In a preliminary study, we reported HIV-1 Tat peptide detection based on an RNA aptamer using optical and potentiometric methods that only focus on an arginine-rich region (Ruslinda et al., 2011). The partial region of the Tat peptide has been successfully detected using a diamond SGFET. This peptide induced a voltage shift of ~40 mV in the negative direction in the FET transfer characteristics. To use this peptide in clinical samples, it is of paramount importance to investigate the effect of a real sample of HIV-1 Tat protein on diamond-FET-based RNA aptamer sensing. HIV-1 Tat consists of 86 amino acids that encompass an acidic and proline-rich N terminus (amino acids 1–21), a cysteine-rich region (amino acids 22–37), a core (amino acids 38–47), a basic region (amino acids 48–57) and a glutamine-rich region (amino acids 58–72) (Arya et al., 1985). Among them, the basic region is important for TAR binding (Weeks et al., 1990) and nuclear localization, and the segment between amino acids 47 and 57 has been used to transport a large variety of materials, including proteins, DNA, drugs, imaging agents, liposomes and nanoparticles, across cell and nuclear membranes (Gupta et al., 2005). In addition, the Tat amino acid sequence has low overall hydrophobicity and a high net positive charge (Shojania and O’Neil, 2006), where the charge derived to HIV-1 Tat protein consists of 21 hydrophilic side chains bringing a large number of positive charges, 5 dicarboxylic amino acids that bring negative charges, and amino acids that are polar, nonpolar and aromatic, which are uncharged. This protein contributes to several pathological symptoms of HIV-1 infection and also plays a critical role in virus replication. The total number of Tat protein amino acids is 86; thus, its size is roughly estimated to be less than 5 nm in diameter.

Here, we report the contribution of HIV-1 Tat protein to the electronic response of a diamond FET-based RNA aptamer to the electronic response of such biosensors. The analytical characteristics of the diamond FET biosensor in terms of sensitivity, stability and reusability have been studied in detail. The reliable use of a real sample of protein by a diamond-FET-based RNA aptamer was demonstrated for the first time, which showed the potential of diamond interfaces for adaption to the clinical monitoring and biological diagnosis.

2. Materials and methods

2.1. Chemicals and oligonucleotides

RNA aptamers were purchased from Sigma-Aldrich Co. The RNA aptamer sequences were 5’-UCGGUGCGAUCCGUUCAUAA-3’-NH2 (probe RNA aptamer) and 5’-GAAGCCUUGAUCCCGA-3’ (aptamer-derived second strand), where underlined residues form bulges in the duplex structure. The recombinant Tat HIV-1 (HIV-1 Tat; 86 amino acids) was purchased from Immuno Diagnostics, Inc., and stored at ~75 °C. The 3′ ends of the probe RNA aptamer were terminated with an amino group. This amino group can covalently immobilize the aptamer to a carboxyl-terminated linker molecule such as terephthalic acid. All other chemicals and solvents used in this experiment were purchased from Kanto Chemical Co. Inc. The buffer used for the experiments consisted of phosphate-buffered solution (PBS) with Tween-20 (0.1% Tween-20), TE buffer solution (Tris–HCl; 4 ml and EDTA; 0.8 ml) and urea solution (8.3 M). Ultrapure water was obtained from a Milipore system.

2.2. Synthesis of partially functionalized diamond surface

Polycrystalline diamonds purchased from Element Six Co. Ltd. were used in this study. These samples are freestanding, transparent diamonds (optical grade) with a thickness of 300 μm and a large grain size (~100 μm) grown by chemical vapor deposition. Their surfaces were sufficiently flat for the fabrication of high performance FETs with a high cutoff frequency (Hiroma et al., 2008). The surfaces used as substrates were H-terminated using a hydrogen plasma. At room temperature, the sheet resistance and carrier concentration of these substrates were 10–20 kΩ/square and 1–3 × 10^{13} cm⁻², respectively, as determined by direct current Hall effect measurements (Song et al., 2006; Kuga et al., 2008). Partial surface amination of the H-terminated diamond was performed by irradiation with UV light in ammonia gas ambient at 100 sccm for 4 h. These procedures were performed at room temperature and atmospheric pressure, allowing the modification to be performed in a short time.

2.3. Diamond FET fabrication

Diamond FETs were fabricated as follows. Source and drain electrodes were deposited onto H-terminated diamond by thermal evaporation using a metal mask consisting of 150 nm-thick Au film. Then Ar ions were implanted (acceleration voltage 25 keV, ion density 2 × 10¹⁴ cm⁻²) through another metal mask to form an insulating region outside the metal electrodes and channel/gate region. The dosed region was highly resistive and did not form graphitic defects (which can be found at ion densities greater than 10¹⁶ cm⁻²) and exhibited the expected electrochemical properties. Wires were bonded to the drain and source electrodes using electroconductive paste and were covered with insulating epoxy resin to protect them from the electrolyte solution. A bulk electrode was not used in this experiment because diamond is an insulating material and the surface channel of the diamond FET was electrically isolated from the bulk like silicon on insulator (SOI). The channel was directly exposed to the electrolyte solution. The length and width of the gate channel were 500 μm and 8 mm, respectively. In each step, the static current–voltage (iDS−VDS) characteristics of the SGFET were measured under a fixed concentration of 1 mM PBS (pH=7) using a Ag/AgCl reference electrode as the gate electrode. iDS was measured as a function of VDS at a constant drain-source voltage (VDS) of ~0.1 V.

2.4. Covalent immobilization of RNA²²⁴

Following the partial amination of the diamond FET, the immobilization of RNA²²⁴ aptamer via terephthalic acid linker molecules was performed on the channel surface of the diamond FET. Each sample was first treated with a 1:1 mixture of 0.1 M N-hydroxysuccinimide (NHS) and 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) for 1 h to activate the
carboxylic functional groups. Then the RNA\textsuperscript{Tat} aptamer was diluted with 3 × sodium saline citrate (SSC), 0.1 M NHS and 0.4 M EDC, to a final concentration of 20 μM, and small droplets of the solution were again deposited manually on the surface. Each sample was incubated at 38 °C for 2 h in a humidified chamber, which was followed by thorough rinsing with PBS with Tween-20 (0.1% Tween-20). The binding of HIV-1 Tat protein with the immobilized probe RNA\textsuperscript{Tat} aptamer were performed at room temperature for 1 h. The concentration of HIV-1 Tat protein was 100 nM in 2 × SSC buffer solution. Following Tat protein binding, the aptamer-derived second strand was introduced to the channel for 1 h at room temperature. The concentration of the second strand of aptamer was 100 nM in 2 × SSC buffer solution. The sample was then rinsed with Tris–HCl buffer solution for 7 min to avoid nonspecific binding. To remove the Tat protein to achieve the regeneration process, urea (8.3 M) solution was used.

3. Results

3.1. Detection of HIV-1 Tat protein using diamond FET-based RNA aptamer

A cross-sectional scheme of the detection of a real sample of HIV-1 Tat protein using a diamond-FET-based RNA aptamer is shown in Fig. 1. Fig. 1b depicts the sensing mechanism of HIV-1 Tat protein on the channel surface using the RNA\textsuperscript{Tat} aptamer as a probe molecule. The RNA\textsuperscript{Tat} aptamer is immobilized on the partially aminated diamond surface via a terephthalic acid linker. The Tat protein is then introduced to the probe, then to the aptamer-derived second strand. In the presence of Tat protein, the probe RNA aptamer and aptamer-derived second strand undergo a conformational change to form a duplex structure, causing a significant shift in the potential gate voltage. Detection using the diamond FET biosensor is based on the change in the charge distribution on the channel surface. When the channel surface is immersed in electrolyte solution, the carrier density on the channel surface is modulated by the field effect of the electric charge of the biomolecules near the solid surface. In a diamond FET, the density of surface holes increases or decreases with the number of biomolecule-derived charges such as positively or negatively charged ions, respectively (Kuga et al., 2008; Ruslinda et al., 2010). This type of FET is known as a solution-gate field-effect transistor (SGFET) (Kawarada et al., 2001; Song et al., 2003) and was first demonstrated on a diamond surface. For instance, when positively charged proteins such as Tat peptide approach the surface, the \(i_{DS}-V_{GS}\) characteristics shift in the negative voltage direction (Ruslinda et al., 2011), corresponding to a drop in current magnitude. A voltage shift in the same direction is expected in the present study, because the HIV-1 Tat protein also has a large number of positive charges.

To prove that the diamond FET functions, \(i_{DS}-V_{DS}\) measurement was carried out beforehand using Ag/AgCl reference electrode in 1 mM PBS buffer solution (pH 7.4). The characteristics show a typical increase and saturation (pinch-off) of the channel current \(i_{DS}\) as the drain-source voltage \(V_{DS}\) increase as shown in Fig. 2. The channel current is also strongly dependent on the gate potential \(V_{GS}\). The FET is normally in the on state. \(i_{DS}\) decreases as the gate potential becomes less negative as expected for p-type surface conductivity. This characteristic proves that the FET based on partially aminated H-terminated diamond is fully functional. The characteristic remains qualitatively the same after RNA\textsuperscript{Tat} aptamer immobilization as well as HIV-1 Tat protein detection, but the overall current is reduced. Without PBS buffer solution, the IV characteristics substantially the same can be obtained as long as we use the electrolyte solution.

3.2. Sensitive detection of HIV-1 Tat protein by diamond FETs

The effect of a real sample of HIV-1 Tat protein on the electronic properties of the diamond-FET-based RNA aptamer is shown in Fig. 3. The transfer characteristics of probe RNA\textsuperscript{Tat} aptamer immobilization (white circles) and Tat protein binding (green triangles, black squares, red circles) were determined at \(V_{GS} = -8 \mu A\) and \(V_{DS} = -0.1 \text{ V}\) for three different concentrations of the Tat protein. The gate potential shifted in the negative direction when Tat protein approached the RNA aptamers on the surface channel of the diamond FET. The sensitivity is defined as the change in surface potential, which reflects the change in drain current. A 91 mV shift in the negative direction was observed upon Tat protein binding (100 nM concentration) as shown in Fig. 3. This result is explained by the fact that HIV-1 Tat protein is rich in positive charges (Shojania and O’Neil, 2006). In addition, quantitative measurements of the diamond-FET-based RNA aptamer at Tat protein concentrations of 10 nM and 1 nM were carried out. The gate potential shifted by an amount that depended on the protein concentration 49 mV at a concentration of 10 nM and 20 mV at a concentration of 1 nM. The detection of nanomolar concentrations of Tat protein is an advantage of using a diamond FET as a biosensor platform. The size of the diamond FET device in our experiment is 0.5 mm × 8 mm whereby the immobilized probe DNA or RNA density is \(10^{11} - 10^{12} \text{ cm}^{-2}\) on the partially aminated channel surface (Kawarada and Ruslinda, 2011). This amount of probe density will create the binding sites on the channel surface approximately \(4 \times 10^{9} - 4 \times 10^{10}\) molecules. In our experiment, the volume of the protein sample was 20 μl, where the solution of 1 nM corresponds to 1.2 × 10\(^{10}\) molecules. These molecules partially occupy the probe sites on the channel surface and provide the voltage shift of 20 mV as shown in Fig. 3. However, when the sample concentration down to 100 pM corresponding to 1.2 × 10\(^{9}\) molecules, the probe sites cannot be occupied enough to make the voltage shift beyond the detection limit. This is owing to...
the number of molecules in solution becomes less than the number of molecules in the binding sites on the channel surface. To overcome the sensitivity limitation, the device miniaturization is expected. We have previously reported the 5 \( \text{mm} \times 80 \text{mm} \) channel SGFET (Song et al., 2007) which is 4 orders of magnitude smaller than the present device and the probe sites on the channel surface are only \( 4 \times 10^7 \)–\( 4 \times 10^8 \) molecules which correspond to 30–300 fM in the sample volume of 20 \( \text{mL} \). If we used the same volume of the protein sample on the miniature device, the same voltage shift can be expected. Hence, the high sensitivity of protein detection could be achieved. Since the isoelectric point (pI) of Tat protein is 9.88, Tat protein is positively charged under this experimental condition (pH \( = 7.4 \)). It is reasonable that Tat protein binding is observed as a shift of the static \( i_{DS} - v_{GS} \) characteristic in the negative direction. Furthermore, the linker is effective for achieving the selective detection of a real sample due to the repulsion force induced by the nonspecific adsorption of RNA aptamers (Ruslinda et al., 2011). Thus, the shift in the positive direction expected from negatively charged molecules such as RNA aptamer was negligible in the present case.

For reference, the response of the diamond FET in the case of RNA aptamer sensing without HIV-1 Tat protein was tested (see Fig. S1 in the supporting information). The interaction between these two RNA aptamers, i.e. the aptamer-derived second strand and the probe RNA\(^{\text{Tat}}\) aptamer in solution, did not generate any signal. The aptamer-derived second strand does not bind to the probe RNA\(^{\text{Tat}}\) aptamer itself, but with the presence of Tat protein both the RNA aptamers and the Tat protein bind to each other in the channel surface. This implies that without the presence of Tat protein, the two aptamers do not bind to each other to form a duplex structure. From this viewpoint, we can conclude that this RNA aptamer is highly specific towards HIV-1 Tat protein. Therefore, no shift in the gate potential was observed.

### 3.3. Reusability of diamond-FET-based RNA aptamer

To investigate the reusability of the diamond-FET-based RNA aptamer, the static characteristics were repeatedly measured to confirm the high selectivity and sensitivity for the detection of HIV-1 Tat protein. Fig. 4a shows the shift in gate potential at \( i_{DS} = -10 \mu \text{A} \) upon Tat protein binding to the RNA aptamer. Urea solution (8.3 M) were used to remove both the Tat protein and the aptamer-derived second strand RNA from the probe RNA\(^{\text{Tat}}\).
aptamer for regeneration. The gate voltage shifted repeatedly between $\sim 20 \text{ mV}$ and $\sim 30 \text{ mV}$ during three cycles of Tat protein binding and regeneration. These oscillations of the gate potential caused by the cycling of binding and regeneration are shown in Fig. 4b and indicate that the functionalized diamond-FET-based RNA aptamer can be used as a reusable biosensor. The positive aspects of our mode of sensing are as follows: the covalent binding is sufficiently stable for repeatable and reproducible biosensing owing to the stable amide bonding; the short distance between the nucleic acids immobilized at the channel surface of the diamond SGFET reflect the interaction between biomolecules in which the Debye length is a major factor; and the capacitance per unit area is higher in the diamond FET than in other FETs. However, the device was still unable to detect low concentrations of the biomolecules. To overcome this problem, a boron delta-doped layer (Edgington et al., 2012) on a diamond SGFET could improve the hydrogen-terminated diamond FET by providing high channel mobility. Moreover, its surface functionalization is less constrained by it not having to maintain its hydrogen, allowing more versatile and tailored surface functionalization for molecular biosensing.

Moreover, our results indicated that the immobilization of probe RNA\textsuperscript{Tat} aptamer on the diamond surface using short linker molecules yielded a diamond FET suitable for detecting either protein or DNA (Yang et al., 2006) because the changes in the biochemical potential were reflected effectively. The sensitivity of FET-based sensors is strongly related to the properties of the channel surface such as channel mobility, capacitance on the gate surface and threshold voltage. However, the changes in binding efficiency caused by the probe RNA\textsuperscript{Tat} aptamer and surface functionalization are the main reason for the detection of Tat protein with high sensitivity. This is because highly sensitive detection can also be achieved by fluorescence observation on a partial region of Tat peptide, as previously reported (Ruslinda et al., 2011), the sensitivity of which is not related to the semiconducting properties of diamond.

4. Discussion

The diamond-FET-based RNA aptamer used for the detection of HIV-1 Tat protein exhibited a shift in the transfer characteristics towards a more negative gate potential. This corresponds to a decrease in the charge carrier density on the surface channel. Since field-effect devices are surface-charge measuring devices, they can in principle measure the intrinsic charge of adsorbed biomolecules or the change in charge due to a DNA-hybridization or protein-binding event (Nebel et al., 2001; Song et al., 2004). However, only changes in the charge density that occur within the Debye length from the interface can be detected. As already been described (Poghosssian et al., 2005; Landheer et al., 2005), the effect of counter-ion condensation will reduce the expected DNA-hybridization signal, particularly in solutions with high ionic strength. Therefore, the expected hybridization signal may be small up to several mV, depending on the density of the immobilized probe DNA and the ionic strength of the solution.

The charge distribution induced by biomolecules on a diamond FET in electrolyte solution can be discussed by considering two capacitances, the electric double-layer capacitance and accumulation layer capacitance, as shown in Fig. 5. The concept of accumulation layer capacitance is similar to that of the proposed inversion layer capacitance in Si MOSFETs due to band bending near the surface (Takagi and Toriumi, 1995). This capacitance density is comparable to the electric double-layer capacitance (1–5 \(\mu\text{F/cm}^2\)). The positive charge induced by HIV-1 Tat protein, \(\Delta Q\text{Tat}\), is located between these two capacitances in the electrolyte solution as shown in Fig. 5. The Tat protein-induced charge distributed in the accumulation layer capacitance induces electrons, which compensate the positive holes. The decrease in the number of holes depends on the size of the accumulation layer capacitance. Therefore, when Tat protein attaches to RNA aptamers on the diamond FET channel, the hole concentration will decrease. The charge density of the accumulation layer capacitance,
which corresponds to the carrier density of the diamond FET channel, can be expressed as (Kawarada and Ruslinda, 2011)

\[ \Delta Q_{\text{acc}} = \Delta Q_{\text{Tat}} \frac{C_{\text{acc}}}{C_{\text{C}} + C_{\text{d}}} = \frac{C_{\text{acc}}}{C_{\text{d}}} \Delta Q_{\text{Tat}} = C_{\text{d}} \Delta Q_{\text{Tat}} / C_{\text{d}} = C_{\text{d}} \Delta Q_{\text{Tat}} / C_{\text{d}} = C_{\text{d}} \frac{v_{gs}}{V_{T}} \]

where \( \Delta Q_{\text{acc}} \) is the decrease in hole carrier density, \( \Delta Q_{\text{Tat}} \) is the Tat-protein-derived charge density, \( C_{\text{acc}} \) is the accumulation layer capacitance density, \( C_{\text{d}} \) is the electric double-layer capacitance density, \( C_{\text{g}} \) is the gate capacitance density (F cm\(^{-2}\)) and \( v_{gs} \) is the shift in gate voltage caused by the electric-double-layer capacitance without the accumulation layer capacitance. In this experiment, when \( v_{gs} \) was 91.6 mV (100 nM concentration), the areal density of charges resulting from protein binding, \( \Delta Q_{\text{Tat}} \), was calculated to be \( 2.86 \times 10^{-12} \) e cm\(^{-2} \) when \( C_{\text{d}} \) was assumed to be \( 5 \) \( \mu \)F/cm\(^2\). At pH 7.4, Tat protein consists of a large number of positive charges due to arginine rich (Yamamoto et al., 2000; Shojaei and O’Neill, 2006). Even above a pI of 9.88, the number of negative charges can be expressed as \( \Delta Q_{\text{Tat}} / C_{\text{d}} \).

\[ K_{\text{a}} = \frac{Q_{\text{Tat}}}{C_{\text{d}}}, \]

\[ K_{\text{a}} = \frac{Q_{\text{Tat}}}{C_{\text{d}}}, \]

5. Conclusions

We have shown that diamond FETs based on RNA aptamers in electrolyte solution exhibit a good FET performance in biologically related environments. The effectiveness of a diamond-FET-based RNA aptamer for the detection of a real sample of HIV-1 Tat protein at concentrations down to 1 nM was demonstrated for the first time. Stable RNA aptamer immobilization was achieved on an aminated diamond surface using carboxyl-terminated tert-ephalic acid, and the reusability of the RNA aptamer as a sensing probe that is only specific to the Tat protein was demonstrated. The simplicity and specificity of this approach has great potential for monitoring in clinical and biological fields. Thus, the solid surface channel of a diamond FET in solution is regarded as an ideal device for protein sensing using an aptamer.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2012.07.048.

References


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