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An Aptameric Graphene Nanosensor for Label-Free Detection of Small-Molecule Biomarkers

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Abstract

This paper presents an aptameric graphene nanosensor for detection of small-molecule biomarkers. To address difficulties in direct detection of small molecules associated with their low molecular weight and electrical charge, we incorporate an aptamer-based competitive affinity assay in a graphene field effect transistor (FET), and demonstrate the utility of the nanosensor with dehydroepiandrosterone sulfate (DHEA-S), a small-molecule steroid hormone, as the target analyte. In the competitive affinity assay, DHEA-S specifically binds to aptamer molecules pre-hybridized to their complementary DNA anchor molecules immobilized on the graphene surface. This results in the competitive release of the strongly charged aptamer from the DNA anchor and hence a change in the conductance of the graphene, which can be measured to achieve the detection of DHEA-S. We present experimental data on the label-free, specific and quantitative detection of DHEA-S at clinically appropriate concentrations with an estimated detection limit of 44.7 nM, and analyze the trend observed in the experiments using molecular binding kinetics theory. These results demonstrate the potential of our nanosensor in the detection of DHEA-S and other small molecules in biomedical applications.

Abstract

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Keywords

aptamer; competitive assay; dehydroepiandrosterone sulfate (DHEA-S); graphene; nanobiosensor; small molecule

1. Introduction

Graphene is a nanomaterial consisting of a monolayer of carbon atoms arranged in two-dimensional hexagonal crystalline form (Novoselov et al. 2004; Novoselov et al. 2005b). The monoatomic structure of graphene offers unique physical properties such as high mechanical strength (Lee et al. 2008) and distinctive electronic properties (Barreiro et al. 2009). In particular, with its charge carriers confined within a one-atom thin layer, graphene exhibits a high carrier mobility, saturation velocity and low charge scattering (Novoselov et al. 2005a; Ponomarenko et al. 2009; Tarruell et al. 2012). Owing to these properties and the high surface-area-to-volume ratio, graphene has been used in the sensitive detection of analytes based on electrochemical (Chen and Hone 2013), optical (Bonaccorso et al. 2010), plasmonic (Kravets et al. 2013) and conductance (Hess et al. 2013) principles. In particular, conductance-based graphene nanosensors have received much attention because of their simple designs and ability to allow sensitive measurements.

In conductance-based sensing, physical or chemical adsorption of charged molecules on or in the vicinity of the sensor surface alters the carrier equilibrium behavior, thereby changing the electrical conductivity of the sensing material (Britnell et al. 2012). With every atom located on the surface and hence conductivity strongly dependent on the surface charge, graphene is a highly effective sensing material to enable sensitive conductance-based detection of analytes.

While conductance-based graphene nanosensors have been developed for detection of analytes in gaseous media (Schedin et al. 2007), their applications in liquid media are still in early stages. In aqueous buffers, graphene has been used as the conducting channel material in field effect transistor (FET) devices. By measurement of changes in conductance of graphene induced by surface doping of ions, graphene FET sensors have been used in the detection of pH or ion concentrations (Ang et al. 2008; Fu et al. 2013; Ohno et al. 2009; Zhu et al. 2015). When functionalized with receptors (e.g., antibodies (Mao et al. 2010) or aptamers (Ohno et al. 2010)), such sensors have also allowed the specific detection of biological analytes such as DNA (Cai et al. 2014; Dong et al. 2010), proteins (Kwon et al.

2012; Ohno et al. 2010) and cells (Hess et al. 2011; Mohanty and Berry 2008). Compared to sensors that rely on enzymatic electrochemical reactions (Shao et al. 2010), affinity sensors do not involve irreversible consumption of the analyte and are not susceptible to interferences from electroactive species, and can in general be more robust and stable. However, such sensors are currently limited to detect highly-charged, large-sized analytes. Conductance-based graphene nanosensors for specific detection of small and weakly-charged analytes, such as organic compounds and peptides, which are challenging as the molecular binding does not directly induce detectable changes in surface charge, have yet to be demonstrated.

This paper presents a conductance-based graphene nanosensor that uses an aptamer-based competitive affinity assay for label-free and specific detection of low-charged small-molecule analytes. The competitive affinity assay exploits analyte binding-induced changes in the conformational structure of an aptamer (Das et al. 2011; Shi et al. 2013; Wang et al. 2009) and is incorporated in a graphene FET sensor embedded in a microfluidic channel. Aptamer molecules are anchored on the graphene surface, and released upon specific binding to target small molecules. As aptamer molecules are highly charged as an electron donor, their release significantly decreases the surface charge, which induces a change in the graphene conductivity to enable the detection of small-molecule analyte. Unlike existing graphene biosensors that rely on direct measurements of equilibrium affinity binding (Cai et al. 2014; Dong et al. 2010; Hess et al. 2011; Kwon et al. 2012; Mohanty and Berry 2008; Ohno et al. 2010), our nanosensor measures the half-time of the aptamer release from the surface to quantify the concentration of small-molecule analytes. The half-time, as a measurand inherent to the kinetics of the molecular binding system, uses the affinity binding-induced conductance change and affords device-to-device uniformity in biosensing that may otherwise be difficult to attain because of variations in the nanosensor fabrication processes. In addition, the embedding of the nanosensor in a microchannel allows for integrated liquid handling for in situ graphene functionalization as well as analyte detection without any off-chip operations.

The utility of our device is demonstrated by detecting dehydroepiandrosterone sulfate (DHEA-S), a diagnostically important steroid biomarker. This small molecule (molecular weight: 426.5 Da) is a building block for sex hormones in the human body; and its concentration in blood relates to metabolism, aging and pathological changes of organs (Baulieu et al. 2000; Lamberts et al. 1997; Lapchak et al. 2000). While accurate measurement of DHEA-S is of great importance to clinical diagnostics, conventional assay methods such as immunofluorescence assay (IFA), radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) (Asif et al. 2006; Bélanger et al. 1989; Lewis et al. 1996) require the labeling of the analyte and typically have low quantification accuracies. In this study, we show that our graphene nanosensor is capable of label-free and specific detection of DHEA-S at well below the sub-micromolar levels as appropriate in clinical tests, and can potentially be used for the detection of other small-molecule analytes in clinical diagnostics.

The principle of competitive affinity assay on graphene (Fig. 1) exploits target-induced change in the conformational structure of a specific aptamer (Das et al. 2011; Shi et al.

2013; Wang et al. 2009). The aptamer, which is a synthetic oligonucleotide that binds to the target analyte with high affinity and specificity (Bunka and Stockley 2006), is used as a molecular recognition element. A short DNA sequence (used as an anchor) complementary to the aptamer is attached to the graphene surface (Fig. 1a), which is then incubated with aptamer solution to generate an aptamer-DNA anchor hybrid layer (Fig. 1b). When exposed to an analyte sample, the aptamer binds with the analyte molecule, thereby folding into a closed T-junction conformation (Fig. 1c) (Das et al. 2011; de-los-Santos-Álvarez et al. 2008; Shi et al. 2013; Wang et al. 2009). This change in the aptamer conformation can disrupt the surface hybridization, releasing the aptamer from the surface-immobilized DNA anchor (Fig. 1d). Since the aptamer, as single-stranded DNA, is highly charged, the release of the aptamer can induce significant changes in graphene conductance. Furthermore, a higher analyte concentration provides a greater binding opportunity, which causes a faster aptamer release from the DNA anchor. Based on this principle, the concentration measurement of low-charged, small-molecule targets can be related to the observation of the time rate of the aptamer release.

2. Materials and methods

2.1. Materials

Annealed copper (Cu) foil (99.8%, 25 μm thick) was purchased from Alfa Aesar (Ward Hill, MA) for chemical vapor deposition (CVD) graphene synthesis. oxidized silicon (Si) wafer (300 nm SiO_2 coated) was obtained from Si-Tech (Topsfield, MA) for device substrate. Polydimethylsiloxane (PDMS, Sylgard-184) was purchased from Dow Corning (Midland, MI) for microfluidic channel fabrication. PASE linker purchased from Life Technologies (Carlsbad, CA), DNA aptamer of DHEA-S (Yang et al. 2012) (selective sequence 5'-CTG CTC TCG GGA CGT GGA TTT TCC GCA TAC GAA GTT GTC CCG AG-3') and 5'-amino group modified DNA anchor (selective sequence 5'- NH_2 -GTC CCG AG-3') acquired from Integrated DNA Technologies (Coralville, IA), were used for graphene functionalization. Target analyte DHEA-S, control analyte DOC, polymethylmethacrylate (PMMA), ethanolamine and PBS as well as other chemicals used in functionalization and experiments were obtained from Sigma Aldrich (St. Louis, MO).

2.2. Device fabrication and biochemical functionalization

Our graphene nanosensor was configured as a liquid-gated graphene FET device (Ohno et al. 2010) (Fig. 2a). A source electrode was connected to the backside of the device substrate (used as a back-gate) to provide a ground potential, while a fixed bias voltage, forming a top-gate voltage (V_{gs}), was applied to the graphene through the liquid. In this standard FET configuration, the electric conductance of graphene, which depends on the charge on the graphene surface, was measured from the drain current (I_{ds}) at a fixed drain voltage (V_{ds}). Details of CVD-graphene synthesis and device fabrication process are described in Supplementary Information, Fig. S1 – S4. Briefly, as shown in Fig. 2b, a sheet of CVD-graphene with a PMMA protective layer was transferred onto an SiO_2/Si substrate connected to the drain and source electrodes (chrome (Cr)/gold (Au) layers deposited on SiO_2). A Cu layer was deposited on the backside of the Si substrate to create a back-gate electrode. A microfluidic channel fabricated of PDMS with inlet and outlet ports was then

bonded to this sensor substrate by oxygen plasma (RIE 800 SOP, Technics. Power: 50 mW, pressure: 250 mTorr, time: 3 s) for fluid handling. A platinum (Pt) wire was inserted into the sample solution within the microchannel to supply the top-gate voltage V_{gs} , and the protective PMMA layer on graphene surface was dissolved by injecting acetone into the microchannel. Photographs of a packaged device and a micrograph of the graphene module are shown in Fig. 2c.

The graphene channel was then biochemically functionalized in situ to realize the sensing principle in Figure 1 (Supplementary Information, Figure S5 for details). To verify the aptamer functionalization, the topography of the graphene (Figure 2d and 2e) was analyzed using atomic force microscopy (AFM, XE-100, Park System). The apparent height of the graphene was seen to have increased by approximately 3 nm after the aptamer functionalization process, and this reflected that aptamer molecules had been captured on the graphene surface.

2.3. Instrumentation

The measurement circuits (Fig. 2a) used a triple DC power supply (E3631A, Agilent) to provide the DC drain voltage V_{ds} , a function generator (33220A, Agilent) to supply the DC top-gate voltage V_{gs} , and a digit multimeter (34410A, Agilent) to measure the drain current I_{ds} . Measurements were automatically controlled through a PC-based Labview program.

3. Results and discussion

3.1. Electrical characterization

As a zero-bandgap semiconductor nanomaterial, the conductance of graphene is described by the transfer characteristics equation (Kim et al. 2010):

$$I_{ds} = \mu(W/L)CV_{ds}(V_{gs} - V_{Dirac}) \quad (1)$$

where C is the top-gate capacitance per unit area, μ is the carrier mobility, W and L are respectively the width and length of the graphene channel, and V_{Dirac} is the voltage indicating Dirac point (I_{ds} achieves the minimum at $V_{gs} = V_{Dirac}$). In aqueous media, molecular binding on a graphene surface creates a self-assembled monolayer (SAM) and forms an electrical double layer (EDL), which is a thin layer at the interface of an electrolyte solution and a charge surface in which counter ions dominate co-ions in concentration (Fujimoto and Awaga 2013). In our device, the EDL can be represented as a parallel-plate capacitor on the graphene surface (Chen et al. 2012), across which a top-gate voltage V_{gs} is applied and controls the carrier density and the electrical conductivity of graphene.

We characterized the electrical properties of the nanosensor with the graphene at the different steps of functionalization, as its surface, starting unfunctionalized (bare graphene surface), was successively coated with 1-pyrenebutanoic acid succinimidyl ester (PASE), DNA anchor and the DNA aptamer specific to DHEA-S (Yang et al. 2012). During the electrical characterization, I_{ds} (with V_{gs} and V_{ds} fixed) was determined by averaging 100

measurements made over a 100-s period. To evaluate the noise level in the signals, standard deviation values of I_{ds} were calculated by:

$$\sigma_{I_{ds}} = \sqrt{\frac{1}{99} \sum_{k=1}^{100} (\bar{I}_{ds} - I_{ds,k})^2}$$

From the detail transfer characteristics (Supplementary Information, Fig. S6a – S6d) and noise level (Fig. S6e – S6h) measurements, it was found that the drain current I_{ds} at a fixed top-gate voltage V_{gs} was directly proportional to the drain voltage V_{ds} , while the noise level $\sigma_{I_{ds}}$ was almost invariant of V_{ds} . This is expected as the carrier scattering in graphene lattice is independent of V_{ds} (Hwang et al. 2007). Thus, a higher V_{ds} was beneficial for the accurate determination of I_{ds} by reducing the influence of noise. Meanwhile, we also observed that the device was prone to lose function when I_{ds} exceeded 40 μ A, possibly because of the electrolytic erosion of the Cr adhesive layer (Fig. S7). Thus, we used a nominal drain current I_{ds} , which was lower than 40 μ A and achieved with a drain voltage of $V_{ds} = 50$ mV, in subsequent experiments for the nanosensor characterization and analyte detection.

Four transfer characteristic curves (Fig. 3a), corresponding to the four graphene functionalization steps, displayed an ambipolar behavior with different Dirac points (Ohno et al. 2010). With the current leakage through the top-gate negligible (Supplementary Information, Fig. S8), it was determined that the nanosensor's transfer characteristics, or the relationship between I_{ds} and V_{gs} , agreed with Eq. (1). The Dirac point shifts, resulting from change in the electric charge on the graphene surface, confirmed the successive surface attachment of the PASE, DNA anchor and aptamer molecules. The results demonstrated that measuring I_{ds} at a fixed V_{gs} can reflect the state of the molecular adsorption on the graphene surface.

In the competitive affinity assay for DHEA-S sensing, we continuously measured the change of I_{ds} (denoted $I_{ds}(t)$) at fixed V_{gs} (below) with respect to the mean of the drain-source current measured when the graphene surface was saturated with aptamer molecules, to monitor the graphene nanosensor responses. As the aptamer-laden graphene varied to the aptamer-free state via aptamer dehybridization from the DNA anchor, $I_{ds}(t)$ decreased/increased with time, eventually reaching an equilibrium value (denoted ΔI_{ds}^{∞}). To enable accurate measurement of $I_{ds}(t)$, we needed an optimal value of V_{gs} that maximized the signal and minimized the noise. To this end, we identified a V_{gs} range from -0.8 to 0 V (Fig. 3b) that provided pronounced ΔI_{ds}^{∞} values between the transfer characteristic curves for the aptamer-free (red) and aptamer-laden graphene (green), and then sought a V_{gs} value in this range optimizing the signal-to-noise ratio (SNR) defined by $SNR = 20 \log(\Delta I_{ds}^{\infty} / \sigma_{I_{ds}})$. Since a detailed understanding of noise mechanisms in graphene electronics is not yet available (Hess et al. 2013), we used the experimentally determined values of $\sigma_{I_{ds}}$ (Supplementary Information, Fig. S6e – S6h) to determine the SNR-optimized V_{gs} value. As shown in Fig. 3c, we obtained two curves depicting SNR vs. V_{gs} using values of $\sigma_{I_{ds}}$ respectively measured from aptamer-free (yellow) and aptamer-laden (blue) states of the graphene surface. Based on these results, we chose $V_{gs} = -0.3$ V, at which the average of the

aptamer-free and aptamer-laden SNR values (dotted curve) was maximized (~ 20.2 dB), in the subsequent experiments.

We then made time-resolved measurements ($V_{gs} = -0.3$ V) of the process of aptamer hybridization to the DNA anchor on graphene (Fig. 3d). Upon incubating the DNA anchor-coated graphene with an aptamer solution, I_{ds} was seen to decrease from $33.2 \mu\text{A}$ and eventually saturated at $23.6 \mu\text{A}$ over time. This observation was consistent with the transfer characteristics shown in Fig. 3b and demonstrated that the aptamer hybridization process was measurable at $V_{gs} = -0.3$ V. Based on these characterization results and the reversibility of DNA hybridization, we chose to use the same top-gate voltage ($V_{gs} = -0.3$ V) in the observation of the reverse process of aptamer hybridization from the DNA anchor during analyte detection.

3.2. Analyte detection

We tested the detection of DHEA-S at concentrations ranging from 0 to 1000 nM (in phosphate-buffered saline (PBS), $\text{pH} = 7.4$) using our graphene nanosensor. In the experiments, performed in triplicates at each DHEA-S concentration, the aptamer-functionalized graphene surface was exposed to a DHEA-S sample solution introduced into the microchannel at a constant flow rate ($1 \mu\text{L}/\text{min}$).

To understand the kinetic process of the competitive affinity assay of DHEA-S, I_{ds} was recorded every 20 s over a 4000-s period for each sample. Experimental results showed that

I_{ds} values, normalized with respect to ΔI_{ds}^{∞} , continuously increased and saturated over time (Fig. 4), reflecting that aptamer molecules were released as they bound to DHEA-S, until being completely removed from the graphene surface. These experimental results also show that a higher DHEA-S concentration led to a shorter equilibration time. This concentration-dependent behavior of aptamer release rate can be attributed to the greater chance for aptamer-analyte interactions at higher DHEA-S concentrations, which caused aptamer molecules to be released faster from the graphene surface.

To demonstrate the specificity of the DHEA-S detection the experiments were repeated using deoxycholic acid sodium salt (DOC, molecular weight: 414.6), a secondary bile acid with a similar molecular structure to DHEA-S, as a control. DOC and mixtures containing equal concentrations of DHEA-S and DOC (Supplementary Information, Figs. S9 – S12) were respectively incubated with the device. The results indicate that the half-time of the nanosensor response was not changed by the presence of DOC, and only depended on the DHEA-S concentration. Thus, the nanosensor was specific to DHEA-S.

3.3. Kinetic model and uncertainly analysis

To better understand our experimental results in Fig. 4, we developed a kinetic model representing the aptamer release process during analyte detection. Denote the aptamer, DNA anchor and target analyte (DHEA-S) by A, B and C, respectively. An analyte molecule specifically binds to the aptamer, disrupting the aptamer-anchor hybrid (denoted AB) (Bunka and Stockley 2006) and releasing a DHEA-S-aptamer complex (denoted AC) into solution (Fig. 1b – 1d). During analyte detection, aptamer molecules are released from the

DNA anchor due to the competitive analyte-aptamer binding as well as the intrinsic

dehybridization: $AB + C \xrightleftharpoons[k_r]{k_f} AC + B$ and $AB \xrightleftharpoons[k_a]{k_d} A + B$, where k_f and k_r are respectively the forward and reverse reaction kinetic constants for the competitive binding, and k_a and k_d are respectively the association and dissociation kinetic constants for the aptamer-DNA anchor hybridization.

Using chemical reaction kinetics theory (Karlsson et al. 1991), the overall rate of change in the concentration of the aptamer-anchor complex, which is directly related to the graphene conductance change, can be represented by the following differential equation (Versteeg et al. 1990):

$$\frac{d[AB]}{dt} = k_r[AC][B] - k_f[AB][C] + k_a[A][B] - k_d[AB] \quad (2)$$

where $[\cdot]$ denotes the concentration of a solution borne species or surface density of a surface-bound species, as appropriate. Assuming that a continuous flow of a DHEAS sample maintains a constant concentration of the analyte while continuously removing the released aptamer and DHEA-S-aptamer complex, we set $[AC] = 0$, $[A] = 0$ and $[C] = c$ (constant) in Eq. (2) (Karlsson et al. 1991). Meanwhile, let x denote the relative surface density of the aptamer-anchor complex, defined as $[AB]$ normalized by the total surface concentration of the free and aptamer-occupied DNA anchor molecules. Then, Eq. (2) can be simplified as:

$$\frac{dx}{dt} = -(k_f c + k_d)x \quad (3)$$

By solving Eq. (3), which governs the kinetic process of aptamer release in the presence of a continuous flow of the analyte, we obtain $x(t) = e^{-(k_f c + k_d)t}$. Therefore, given sufficiently long time and in the absence of free aptamer molecules in the DHEA-S sample, the surface-anchored aptamer molecules will be completely removed by the sample flow. Furthermore, the time dependence of the measurement signal, related to x by $\Delta I_{ds}(t)/\Delta I_{ds}^\infty = 1 - x(t)$, is given by:

$$\Delta I_{ds}(t)/\Delta I_{ds}^\infty = 1 - e^{-(k_f c + k_d)t} \quad (4)$$

Thus, the half-time (denoted $t_{1/2}$) of the aptamer dehybridization process, defined by $\Delta I_{ds}(t_{1/2})/\Delta I_{ds}^\infty = 1/2$, is given by:

$$t_{1/2} = \ln 2 / (k_f c + k_d) \quad (5)$$

Eq. (4) were fitted to the experimental data, with the triplicated results shown as solid curves in each panel of Fig. 4, and the intersections of these curves with the line $\Delta I_{ds}/\Delta I_{ds}^\infty = 1/2$ allowed determination of the half-time values (Fig. 4). It can be seen that Eq. (4) provided an excellent representation of the experimental data, the half-time results thus obtained were plotted against the DHEA-S concentration in Fig. 5. As the DHEA-S concentration varied

from 0 to 1000 nM, the average half-time (denoted $\bar{t}_{1/2}$) from the triplicate experiments was found to decrease from 712.5 s to 205.8 s, in agreement with Eq. (5). By fitting Eq. (5) to the experimentally determined $\bar{t}_{1/2}$ values, we obtained a calibration curve (solid curve in Fig. 5) with the parameters $k_f = 3.892 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$ and $k_d = 9.979 \times 10^{-4} \text{ s}^{-1}$, which could be used to calculate analyte concentrations from the measured aptamer release half-time, $t_{1/2}$.

The kinetic model can be used to estimate measurement uncertainties, thereby determining the device's limit of detection (LOD), i.e., the smallest measurable concentration of the analyte. First, the uncertainty in $t_{1/2}$ can be estimated by the pooled standard deviation (Olofsson et al. 1981):

$$\sigma_{t_{1/2}} = \sqrt{\frac{1}{7} \sum_{i=1}^7 \left\{ \frac{1}{2} \sum_{j=1}^3 [\bar{t}_{1/2}(c_i) - t_{1/2}(c_i, j)]^2 \right\}}$$

where $t_{1/2}(c_i, j)$ is the half-time determined at DHEA-S concentration c_i (respectively equal to 0, 2.5, 10, 25, 100, 250 and 1000 nM for $i = 1, 2, 3, 4, 5, 6$ and 7) via triplicate

experiments ($j = 1, 2$ and 3), and $\bar{t}_{1/2}(c_i) = \frac{1}{3} \sum_{j=1}^3 t_{1/2}(c_i, j)$ is the mean half-time at c_i . This yields $\sigma_{t_{1/2}} = 40.3 \text{ s}$ from the experimental data (Supplementary Information). To assess the uncertainty in the measurement of low concentrations of the analyte, we linearize Eq. (5) at $c = 0$ to obtain $c = -k_d^2 t_{1/2} / (k_f \ln 2)$. Using the kinetic parameter values obtained above, we can estimate the standard deviation in the analyte concentration: $\sigma_c = k_d^2 \sigma_{t_{1/2}} / (k_f \ln 2) \approx 14.9 \text{ nM}$. Therefore, it is determined that for analyte measurements, the limit of detection is given by $3\sigma_c \approx 44.7 \text{ nM}$ (Homola 2008; Piliarik and Homola 2009). This LOD value, achieved via label-free, kinetically based measurements, is about one order of magnitude lower than DHEA-S concentration levels in clinical settings (Higashi et al. 2001; Lewis et al. 1996; Vedhara et al. 2002).

4. Conclusion

We presented a conductance-based graphene nanosensor capable of label-free and specific detection of small-molecule biomarkers. Graphene was used as a sensing material to provide the high sensitivity in electrical conductance measurements. To achieve the label-free sensing of low-charged small molecules, we adopted an aptamer-based competitive affinity assay in which an analyte-specific aptamer was captured via hybridization by a surface immobilized complementary DNA anchor. When the analyte is introduced, its binding with the aptamer will disrupt the aptamer-anchor hybrid on graphene surface. As a result, highly-charged aptamer molecules are released from the graphene surface, inducing changes in the electrical conductance of the graphene. As a proof of principle, the graphene nanosensor was tested with dehydroepiandrosterone sulfate (DHEA-S), a small-molecule steroid hormone. Experimental results showed that the aptamer release rate is strongly correlated to DHEA-S concentration and that the device, with an estimated detection limit of 44.7 nM, was capable of specific and quantitative detection of DHEA-S at concentrations appropriate for clinical diagnostics (Lamberts et al. 1997). Furthermore, a theoretical model was developed to understand the mechanism of the aptamer-based competitive affinity assay in the device and

explain the trend observed in the experimental data. In conclusion, our graphene nanosensor can be potentially used in the specific, quantitative and label-free detection of low-charged small-molecule biomarkers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary information

Supplementary information associated with this article is separately uploaded.

Highlights

An aptamer-based graphene FET biosensor was developed for the label-free and specific detection of low-charged small-molecule analytes.

The device embedded in a microfluidic channel enables in situ liquid handling without off-chip operations.

A competitive affinity bioassay for quantitative measurement of small-molecule analytes was proposed and demonstrated.

The utility of small molecule bioassay was demonstrated on our device by detecting a diagnostically important biomarker, dehydroepiandrosterone sulfate (DHEA-S).

By measuring the half-time of aptamer release from the DNA anchor, we demonstrated the detection of DHEA-S at physiologically relevant concentrations with a limit of detection (LOD) of down to 44.7 nM.

Fig. 1.

Principle of the graphene nanosensor for small molecule detection. (a) The sensing surface is prepared through complementary hybridization between aptamer and the graphene immobilized DNA anchor. Sensing mechanism: (b) Aptamer molecules hybridized to the DNA anchor can specifically bind to target small molecules (DHEA-S) in sample solution. (c) This specific binding changes the conformation of aptamer, and (d) disrupts the aptamer-anchor hybridization, inducing the release of the aptamer from the graphene surface.

Fig. 2.

Design, fabrication and functionalization of the graphene nanosensor. (a) Configuration of the device as a liquid-gated graphene FET. (b) The device consists of a CVD-graphene conducting channel, a microfluidic channel and electrodes. (c) Photographs of a packaged device and a micrograph of the source and drain electrodes connected by graphene. (d) AFM image of pristine graphene. (e) AFM image of aptamer-functionalized graphene. (The sharp peaks reflect artifacts that were likely ethanolamine residues from PASE deactivation.)

Fig. 3.

Electrical characterization of the graphene nanosensor. (a) Transfer characteristic curves (drain current I_{ds} vs. top-gate voltage V_{gs}) obtained at $V_{ds} = 50$ mV, as bare graphene (blue) was successively functionalized with PASE (orange), DNA anchor (red) and aptamer (green). (b) A magnified view of the transfer characteristic curves for V_{gs} ranging from -0.8 to 0 V in (a). $x(t) = 0$ and $x(t) = 1$ respectively indicate the fraction of surface DNA anchor occupied by aptamer before and after hybridization. The error bars in (a) and (b) were determined from the observed maximum and minimum values of I_{ds} , at a given V_{gs} . (c) Signal-to-noise ratio (SNR) as a function of V_{gs} for aptamer-free graphene (yellow), aptamer-laden graphene (blue) and the average (dotted curve). (d) The time course of I_{ds} upon introduction of the aptamer to the DNA anchor-coated graphene at $V_{gs} = -0.3$ V. The current decreased from 33.2 μA , reaching a steady-state value of 23.6 μA as reflected in (b).

Fig. 4.

Measurements of the sensor response over time while a DHEA-S sample solution at a given concentration continuously flowed on the graphene nanosensor surface: $I_{ds}(t)$ normalized by the corresponding final equilibrium value ΔI_{ds}^{∞} at (a) $c = 0$ nM, (b) $c = 2.5$ nM, (c) $c = 10$ nM, (d) $c = 25$ nM, (e) $c = 100$ nM, (f) $c = 250$ nM, and (g) $c = 1000$ nM. Triplicate measurements at each concentration were presented with dots in different colors (red, green and yellow). Fitting of Eq. (4) to the data from each of the triplicate experiments at each DHEA-S concentration was shown as solid curves in black, which can be seen to almost coincide in the figures.

Fig. 5. Measured half-time of aptamer release from the DNA anchor as the DHEA-S analyte was introduced in continuous flow at varying concentrations. The solid curve represents Eq. (5) fitted to the experimentally determined average half-time (diamonds).