The Electrical Detection of Lead Ions Using Gold-Nanoparticle- and DNAzyme-Functionalized Graphene Device

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As a result of industrialization, lead contamination in human environment is an increasingly prevalent health hazard. Even at a low-dose exposure, lead can adversely affect the functions of central nervous system, hematopoietic system, and kidney, causing various severe or fatal consequences (especially in children).[1] The maximum level of lead in drinking water permitted by the United States Environmental Protection Agency is 15 μg/L (~72 nM) (Source: http://www.epa.gov/safewater/contaminants/index.html). Therefore, developing techniques to detect lead at trace levels is of paramount importance. Atomic absorption/emission spectrometry[2] and inductively coupled plasma mass spectrometry[3] have been used to detect lead in aqueous media. But these techniques require sophisticated equipment and/or sample treatment. Electrochemical,[4] colorimetric[5] and fluorescent[6] methods have also been developed with sensitivity ranging from several nM to several 100 nM.[7] It is of high interest to develop new methods in order to further lower the detection limit.

In the past two decades, the zero- or one-dimensional functionalized nanomaterials (e.g., nanoparticles, quantum dots, carbon nanotubes, silicon nanowires) have been the main thrusts for advanced sensor developments.[8] Owing to its extraordinary electronic, chemical, mechanical, optical and structural properties, graphene (a two-dimensional single-atom-thick carbon sheet) has recently attracted increasing attention for the development of various biological and chemical sensors[9] based on different detection modalities including electrochemical,[10] optical,[11] and particularly electrical detection.[12] In this work, we demonstrate a graphene-based electronic sensor for label-free detection of lead (Pb\(^{2+}\)) ions with high sensitivity (lower detection limit ~ 0.02 nM) and high selectivity, using Pb\(^{2+}\)-dependent DNAzyme as the recognition element.

As schematically shown in Figure 1, the device is configured as a liquid-gated field-effect transistor (FET) with graphene film (10 × 2 mm) grown by chemical vapor deposition (CVD) as the conducting (sensing) channel. The source and drain electrodes were made by conductive silver paint. Silicon rubber was used to insulate the electrodes and form a reaction/recording chamber (10 × 5 mm). The graphene was decorated with gold nanoparticles (AuNPs) which serve as the anchoring sites to covalently immobilize thiolated DNAzyme molecules. DNAzymes are a new class of enzyme family. Compared to protein and RNA enzymes, DNAzymes are less expensive, more stable, and can be derived to specifically against a wide range of targets using in vitro selection process - a simple yet powerful technique.[13]

A Pb\(^{2+}\)-dependent DNAzyme, which is a double-stranded DNA consisting of an enzymatic strand (17E) and a thiolated substrate strand (17S), was used here as the recognition element. Upon binding with Pb\(^{2+}\) ion, the enzymatic strand cleaves the substrate strand.[13b] As the consequence, the enzymatic strand and the un-thiolated portion of the substrate strand diffuse away, leaving the thiolated fragment of the substrate strand on the AuNP. This alters the original electronic coupling between the charged DNAzyme complex and the graphene.

The CVD-grown graphene films contain single-layer and few-layer domains. The Raman spectrum of a typical graphene

![Figure 1](image-url)
film presented in Figure 2A exhibits the characteristic D, G and 2D bands. And the intensity ratio between 2D and G bands indicates that the region of the measurement has 2-3 graphene layers. Gold nanoparticles (AuNPs) were readily decorated onto graphene film via spontaneous catalyst-free in-situ reduction. [14] As shown by scanning electron microscopy (SEM), the AuNPs are uniformly distributed on the graphene surface and ∼200 nm in diameter (Figure 2B). Many DNAzyme molecules can be anchored onto a single AuNP.

Graphene exhibits ambipolar field-effect characteristics, i.e., the type of charge carrier (hole or electron) and its density can be modulated by the gate voltage - \( V_g \) (Figure 3). The hole conduction at more negative voltages and electron conduction at more positive voltages transits at the Dirac point - the minimum conduction point where the conduction and valence bands meet (\( V_g,_{\text{min}} \)). Doping holes (p-doping) or electrons (n-doping) to graphene will causes right or left shift of \( V_g,_{\text{min}} \). [15] As show in Figure 3A, the transfer curve (source-drain current vs. gate voltage) and thus its Dirac point is largely shifted after deposition of AuNPs (reduced from HAuCl₄), indicative of p-type doping effect. This is in agreement with the previous reports. [14, 16] It has been demonstrated that DNA molecules can impose strong n-doping to graphene (thus left-shift of Dirac point). [17] Consistently, significant left-shift of \( V_g,_{\text{min}} \) (n-doping) is observed after conjugation of DNAzyme molecules onto AuNPs. After incubation with buffer solution containing 100 nM Pb²⁺ for 20 minutes and subsequent refreshing with Pb²⁺-free buffer solution, \( V_g,_{\text{min}} \) shifts back to right, indicating the alleviation of n-doping. This is expected because Pb²⁺ ion activates the self-cleavage of DNAzyme and consequently causes release of two fragment strands.

Pb²⁺ ions do not cause Dirac point shift in bare graphene devices (without AuNPs), indicating that the observed Pb²⁺ induced responses in Figure 3A is not due to interaction between Pb²⁺ ions and graphene (Figure S1A of the Supporting Information (SI)). Physical adsorption of DNAzyme molecules onto bare graphene via pi-pi interaction [18] (by overnight incubation with 5 μM DNAzyme solution) can also induce left-shift of Dirac point as the result of n-doping (Figure S1B of the SI). However, subsequent addition of Pb²⁺ ions to DNAzyme adsorbed bare-graphene devices causes no further shift of Dirac point. This can be explained that the DNAzyme molecules absorbed onto the graphene surface denature from their native active form because of the strong pi-pi interaction with graphene surface. These experiments demonstrate that the critical roles of AuNPs in enhancing the device sensitivity and preserving the function of DNAzyme molecules.

As shown in the Figure 3B, DNAzyme-AuNP functionalized graphene devices are responsive to Pb²⁺ ions in dose-dependent manner, i.e., the Dirac point shifts to a more positive voltages at a higher Pb²⁺ concentration. In contrast, other divalent metal ions even in a high concentration at 1 mM are not able to trigger significant responses (Figure S1C of the SI). In addition, when the devices with nonspecific (random-sequence) double-stranded DNA (NSDNA) functionalized onto AuNPs were challenged with Pb²⁺ ions, no appreciable alteration in Dirac point was observed (Figure S1D of the SI).

![Figure 2: Raman spectrum of CVD graphene (A), and SEM images before (B) and after (C) decoration of AuNPs. The scale bars in B and C are 10 μm. The inset of C depicts individual AuNPs with a scale bar = 200 nm.](image)

![Figure 3: A) The transfer curves (source-drain current vs liquid gate-voltage) of the bare graphene device, AuNP-decorated graphene device, and DNAzyme–AuNP complex decorated device before and after reaction with 100 nM Pb²⁺. B) The transfer curves after the DNAzyme–AuNP complex decorated device reacted with 0, 0.1, 1, 10, and 100 nM Pb²⁺. The arrow indicates the direction of concentration increase. In both (A) and (B), the HEPEs buffer is used as the electrolyte on top of the graphene device.](image)
The dose responses of Dirac point shift to different Pb²⁺ concentrations are presented in Figure 4A. Each data point is averaged from 3 different bare graphene, AuNP decorated graphene (AuNP-graphene), and DNAzyme–AuNP complex decorated devices, to various Pb²⁺ concentrations. As demonstrated in Figure 4B, increasing incubation time which allows more sufficient interaction between Pb²⁺ ions and DNAzymes leads to larger shift of Dirac point. However, 20 min incubation, which gives the signal-to-noise-ratio (S/N) of Dirac point shift (mean magnitude divided by standard deviation) of 5.11, appears to be optimal time because S/N doesn’t improve much by further time increase.

In summary, we have fabricated a novel electronic lead sensor based on CVD grown transistor, taking advantage of the extraordinary electrical properties of graphene including high conductivity due to large carrier mobility and capacity, high sensitivity to chemical doping, and unique ambipolar field effect. Comparing with other methods, this approach offers several advantages. First, such nanoelectronic detection is simple, label-free, and low-cost. Secondly, it is extremely sensitive (detection limit of 20 pM Pb²⁺) as several orders lower than that of other approaches, e.g., optical detection[7a]. Finally, using Pb²⁺-dependent DNAzyme as the recognition element, the detection is highly specific.

Experimental Section

Materials: All DNAs were synthesized by iDNA Biotechnology Pte Ltd (Singapore). The sequences of DNAzyme and non-specific DNA (NSDNA) are shown in Table 1. All chemicals were purchased from Sigma Aldrich (USA) except that HAuCl₄ was purchased from Alfa Aesar (USA).

Growth of Graphene: The graphene films were synthesized by chemical vapor deposition using copper foils as the growth substrate (2 × 2 cm). Argon and hydrogen flow into the growth tube at the rate of 200 sccm and 100 sccm respectively as the temperature rised from room temperature to 950 °C, then methane was added to the gas mixture at 15 sccm. After 10 mins, the tube was cooled down to room temperature under argon atmosphere.

Characterizations: The Raman spectrum of graphene was taken by JY T64000 (Horiba Jobin Yvon, France) at 532 nm laser with a power of 2 mW and a scan time of 10 s. Field emission scanning electron microscopy (JEOL 7001F FESEM) was used to take the SEM images.

The Device Fabrication: The graphene film on copper foil was spin-coated with a thin layer of poly (methyl methacrylate) (PMMA) followed by cutting into ∼10 × 2 mm pieces and etching away the copper substrate using Ni etchant type TFB (from Transene Company Inc., USA) for 2–4 h. The detached PMMA-coated graphene film was transferred to a glass substrate, followed by removing PMMA with acetone. Subsequently, silver paint was used to make the source and drain electrodes at the opposite ends of the graphene film, and silicon rubber (Dow Croning 3140) was to insulate the electrodes and form a reaction chamber (10 × 5 mm). AuNPs were deposited by immersing the electrically grounded graphene device in HAuCl₄ (10 mM) for 20 min. The two strands (17E and thiolated-17S) of the DNAzyme were transferred to a glass substrate, followed by removing PMMA with acetone. Subsequently, silver paint was used to make the source and drain electrodes at the opposite ends of the graphene film, and silicon rubber (Dow Croning 3140) was to insulate the electrodes and form a reaction chamber (10 × 5 mm). AuNPs were deposited by immersing the electrically grounded graphene device in HAuCl₄ (10 mM) for 20 min. The two strands (17E and thiolated-17S) of the DNAzyme were

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To more accurately determine the lower detection limit, we tested our device in the presence of Pb²⁺ at an ultralow concentration (20 pM) with different incubation durations. As demonstrated in Figure 4B, increasing incubation time which allows more sufficient interaction between Pb²⁺ ions and DNAzymes leads to larger shift of Dirac point. However, 20 min incubation, which gives the signal-to-noise-ratio (S/N) of Dirac point shift (mean magnitude divided by standard deviation) of 5.11, appears to be optimal time because S/N doesn’t improve much by further time increase.

In summary, we have fabricated a novel electronic lead sensor based on CVD grown transistor, taking advantage of the extraordinary electrical properties of graphene including high conductivity due to large carrier mobility and capacity, high sensitivity to chemical doping, and unique ambipolar field effect. Comparing with other methods, this approach offers several advantages. First, such nanoelectronic detection is simple, label-free, and low-cost. Secondly, it is extremely sensitive (detection limit of 20 pM Pb²⁺) as several orders lower than that of other approaches, e.g., optical detection[7a]. Finally, using Pb²⁺-dependent DNAzyme as the recognition element, the detection is highly specific.
synthesized separately. They (both 5 μM) were hybridized to thiolated and double-stranded DNAzyme in pH 7.0 buffer containing 10 mM Tris and 1 M NaCl, with heating to 90 °C for 3 mins followed by cooling to room temperature slowly. Finally, the AuNP decorated graphene devices were incubated with the obtained DNAzyme solution for 2 h to allow immobilization of DNAzyme molecules onto AuNPs. The devices were rinsed with HEPEs buffer (pH 7.0) to remove non-specifically adsorbed DNAzyme molecules.

Electrochemical Measurement: HEPEs buffer (containing 50 mM HEPEs and 100 mM NaNO₃, pH 7.0) was used as the electrolyte in the experiments. The transfer functions of graphene devices were measured by a semiconductor device analyzer (B1500A, Agilent technologies). The voltage between the source and drain electrodes (Vsd) was fixed at 200 mV while the liquid-gate voltage (Vg) was applied via an Ag/AgCl electrode immersed in the buffer solution.

Lead Detection: Pb²⁺ ions were added to the buffer immersed functionalized graphene device to defined final concentrations for 20 min incubation time. The device was then rinsed with the buffer to remove released DNA strands from the self-cleaved DNAzyme. The transfer functions of the graphene devices were measured before and after Pb²⁺ addition in Pb²⁺ free buffer.

Supporting Information
Supporting Information is available from the Wiley Online library or from the author.

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