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**ABSTRACT:** The detection of lead ion  $(Pb^{2+})$  contamination in aqueous media is relevant for preventing endemic health issues as well as damage to cognitive and physical health. Existing home kit tests are unable to achieve clinically relevant sensitivity and specificity. Here, a label-free graphene field-effect transistor sensor for detecting  $Pb^{2+}$  at the femtomolar (fM) level, discriminating between confounding ions, is reported. The sensing principle is based on electrically monitoring  $Pb^{2+}$ -binding-mediated conformational changes of a specific aptamer tethered to graphene, modeled through the Hills–Langmuir mechanism. A *record sensitivity*—through a limit of detection of ~61 fM, for  $Pb^{2+}$  was demonstrated. For model verification, *specific* discrimination of  $Pb^{2+}$  from other ions at the 1 picomolar (pM) level was shown. The reported work provides motivation for development of portable, label-free, point-of-care devices with *both* high specificity and sensitivity.

KEYWORDS: lead ions, graphene, aptamer, field-effect transistor, incubation, Hills-Langmuir mechanism, specificity, sensitivity

# ■ INTRODUCTION

Lead exposure is a serious health concern with detrimental effects on public health<sup>1-3</sup> and through endemic disease outbreaks.4 <sup>-7</sup> A lead concentration more than 15 ppb (~72 nM) in drinking water and larger than 100  $\mu$ g/L (0.48  $\mu$ M) in blood could lead to pernicious outcomes such as stunted human growth and development.8 Consequently, there is substantial motivation for lead ion  $(Pb^{2+})$  detection. The conventional methods of detection, for example, inductively coupled plasma mass spectrometry (ICP-MS), atomic emission/absorption spectroscopy, fluorescent sensing,<sup>9-11</sup> and so forth., while accurate, involve costly instrumentation.<sup>3,12,13</sup> Alternately, home kit utilizing colorimetric schemes are unreliable and have a poor limit of detection (LoD), typically ~10 mg/L (~48  $\mu$ M).<sup>1</sup> Given such issues, portable electrochemical sensors, conjugated with Pb<sup>2+</sup>-specific aptamer  $(Ap)^{14-16}$  and relying on sensor surface interactions, promise an attractive alternative for improved LoD. However, nonspecific interactions between confounding ions must be prevented to reduce the chances of false reporting.

Considering all such aspects, we propose an Ap (short-chain ssDNA)-incorporated graphene field-effect transistor (GFET)based device for Pb<sup>2+</sup> detection.<sup>14–19</sup> A top electrolyte gating scheme was used for the improved efficiency.<sup>20–23</sup> The graphene layer in the GFET was functionalized with specific Ap receptor probes.<sup>14–27</sup> The binding of the target Pb<sup>2+</sup> to the Ap is transduced into modulations of the electrical current through the GFET. Indeed graphene and other 2D materials<sup>28,29</sup> have been extensively deployed for heavy metal detection,<sup>30</sup> in FET-based sensors, as indicated in many reviews.<sup>31–34</sup> However, much less work<sup>16</sup> has been performed

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**Figure 1.** (a) Schematic of the utilized graphene field-effect transistor (GFET) configuration with source (S), drain (D), and gate (G) electrodes for the current (*I*)-voltage (*V*) measurements. The analyte consisting of various Pb<sup>2+</sup> concentration/s is placed in contact with the graphene (transferred onto a SiO<sub>2</sub>/Si substrate). (b) Aptamer (Ap) immobilization on the graphene surface through a PBASE linker. (c) Unbound Ap (*left*) yields an Ap—Pb<sup>2+</sup> G-quadruplex (APG)-*right*, when Pb<sup>2+</sup> is trapped in between the parallel stacked G-quadruplex planes. The APG plays a role in *p*-doping the graphene channel. (d) Pb<sup>2+</sup> detection mechanism involves the APG formation that is detected as a shift in the Dirac voltage:  $\Delta V_D$ , in the  $I_{ds}-V_g$  characteristics.

and reported with regard to specific and sensitive  $\mathrm{Pb}^{2+}$  detection.

We demonstrate how optimization of sensing protocols yields ultrasensitive lead ion concentration: [Pb2+], specific detection, at the femtomolar (fM) level significantly improving, by orders of magnitude, extant reported values.<sup>14-18</sup> Furthermore, the specificity was substantially enhanced to the picomolar (pM) level. Comparing to earlier GFET related work, it was indicated that the use of 8-17 nucleotide (NT) DNAzyme<sup>16,17</sup> (with dsDNA) for Pb<sup>2+</sup> detection—enabled through the cleavage of one strand in the presence of Pb<sup>2+</sup>, yielded an LoD of  $\sim 0.2^{16}$  and  $\sim 0.02$  nM<sup>17</sup> with Aunanoparticle decorated GFET, with specificity at 0.5<sup>16</sup> and 0.1 nM,<sup>17</sup> respectively. While DNAzymes show strong Pb<sup>2+</sup> affinity, their large size and related structural rigidity<sup>38</sup> make lower LoD difficult. In other studies,<sup>14,15</sup> a guanine (G)enriched Ap placed on the graphene surface was reported to have an LoD of 2  $\mu M^{15}$  with a SiO<sub>2</sub> back-gated GFET and an LoD of ~0.8  $nM^{14}$  of  $Pb^{2+}$  on an  $HfO_2\mbox{-gated}$  GFET, with specificity ~48 nM.<sup>14</sup>

We show that the LoD and the specificity for  $[Pb^{2+}]$  detection may be improved significantly by *orders of magnitude*. We adapted a G-quadruplex structured Ap-based modality for label-free Pb<sup>2+</sup> detection, yielding LoD as low as ~ 61 fM and specific determination of  $[Pb^{2+}]$  at 1 pM. The LoD was found to be critically dependent on the incubation time, which regulates the amount of immobilized Ap on the graphene surface. An optimal sensing protocol was determined through extensive experimentation. Furthermore, the sensitivity was related to the doping-induced carrier density  $(n_0)$  and carrier

mobility ( $\mu$ ) of the graphene, arising from an Ap-Pb<sup>2+</sup>-Gquadruplex (APG) formation.

## EXPERIMENTAL SECTION

The GFET fabrication and details on experimental measurements, as indicated in Figure 1a, are further discussed in the Methods and Supporting Information (Sections S1 and S2). Briefly, 1-pyrenebutanoic acid N-hydroxysuccinimidyl (NHS) ester (PBASE, Anaspec, Inc.) was initially immobilized on the graphene surface. The pyrene group in PBASE is attached to the graphene surface via  $\pi$ - $\pi$  interactions (Figure 1b). Subsequently, the Ap (5'-GGGTGGGTGGGTGGGT/-NH<sub>2</sub>-3', from IDT, Inc.) was linked to the PBASE<sup>35,36</sup> (Figure 1b—also explained in Section S3 of the Supporting Information).

The Ap was selected on the basis of (i) the affinity of nucleobases with Pb<sup>2+</sup>, as well as its (ii) size, and (iii) extent of dynamic structure sensitive changes on binding to the Pb<sup>2+</sup>. The Pb<sup>2+</sup> ion shows stronger affinity toward N7/(C6)O of the G while the binding with cytosine (C), adenine (A), and thymine (T) is weaker, even compared to the PBASE-related phosphodiester linkage.<sup>37–39</sup> Consequently, a ssDNAbased Ap with multiple G in the sequence was expected to enhance the Ap–Pb<sup>2+</sup>-binding affinity, with low affinity to other ions (*e.g.*, Ca<sup>2+</sup>, Co<sup>2+</sup>, Al<sup>3+</sup>, Fe<sup>3+</sup>, *etc.*) found in aqueous environments.<sup>14,40</sup> The related interaction between the amine group of the Ap with the NHS ester<sup>14–17</sup> is indicated in Figure 1b.

The PBASE-graphene platform functionalized with the chosen Ap facilitates the formation of a specific APG (Ap $-Pb^{2+}-G$ -quadruplex) structure—consisting of three planar arrays of four G NTs with Pb<sup>2+</sup> in the cavity<sup>39,40</sup> (Figure 1c). The APG is expected to induce positive charge carriers into the graphene surface channel, due to a conformational transformation arising from the Pb<sup>2+</sup> binding that brings the negatively charged G species closer to the surface and



**Figure 2.** (a) Variation of the  $I_{ds}-V_g$  characteristics of the GFET, as a function of the preparation, for Pb<sup>2+</sup> detection, comparing (i) pristine graphene, with that after (ii) attachment of the PBASE linker onto the graphene: purple, followed by (iii) addition of the Ap (10  $\mu$ M): blue, and finally (iv) ethanolamine (EA) addition: green. (b) Change in the  $I_{ds}-V_g$  characteristics as a function of Pb<sup>2+</sup> concentration ([Pb<sup>2+</sup>]) in the range of 100 aM to 100 nM. The ( $V_g$ )<sub>min</sub> is termed the Dirac voltage ( $V_D$ ). (c) Change in the  $V_D$  with [Pb<sup>2+</sup>]. (d) Determined charge carrier density  $n_0$ : red triangles, and the hole mobility ( $\mu$ ): black open circles, as a function of the estimated [Pb<sup>2+</sup>].

forms the basis for the sensor response.<sup>14</sup> Due to its small size (16 NTs) and binding mediated dynamic folding, the chosen Ap enables  $Pb^{2+}$  detection in variable environments, for example, background electrolyte concentration/s. Subsequent to Ap immobilization, the graphene surface was treated by ethanolamine (EA) to passivate the unlinked PBASE NHS groups. The electrical measurements were performed with the GFET in aqueous solutions with different  $Pb^{2+}$  concentration ( $[Pb^{2+}]$ ), as described in further detail in the Methods section. Between and during each measurement step, the sensor was covered with wax paper to minimize the environmental impact. Moreover, given that the graphene is covered by a liquid, we would expect that the direct influencing by the ambient species would be small.

# RESULTS AND DISCUSSION

The underlying mechanism related to FET-based detection is broadly understood<sup>14-34,41,42</sup> and involves the modulation of the electrical current  $(I_{ds})$  in the graphene channel between the drain and source electrodes, by the sensing moiety, such as the Pb<sup>2+</sup> ion—which has been considered here as attached to the Ap yielding the APG. When there is no attached  $Pb^{2+}$ , there would be a particular gate voltage  $(V_g)$  where the  $I_{ds}$  may be tuned to a minimum: blue trace in Figure 1d. Alternately, when there is attachment of Pb<sup>2+</sup>, manifested through the APG formation, a different  $V_{\rm g}$  would be required to indicate the  $I_{\rm ds}$ minimum: green trace in Figure 1d. The related  $V_g$  yielding the minimum  $I_{ds}$  is termed the Dirac voltage (=  $V_D$ ). The difference of the  $V_{\rm g}$  in both the cases is indicated through the  $\Delta V_{\rm D}$ . A positive (/negative) value of the  $\Delta V_{\rm D}$  may be obtained to the presence of positive (/negative)  $n_0$  in the graphene. The  $n_0$  may be correlated to the amount of APG formation, as proportional to the present  $[Pb^{2+}]$ .

However, given the extreme responsivity of the graphene surface, it is important to monitor the variation of the  $V_{\rm D}$ arising just from the preparation of the GFET toward Pb<sup>2+</sup> detection (Figure 2a). We outline the related modulations of the electrical signals. In sequence, (i) a positive shift of  $V_{\rm D} \sim$ 0.25 V was observed after attaching the PBASE linker onto the graphene, due to the positive charge transfer from the PBASE to the graphene,<sup>43</sup> (ii) the addition of the Ap (10  $\mu$ M, diluted in 1× PBS: phosphate-buffered saline solution) induced an opposite/negative  $V_{\rm D}$  shift of ~-0.1 V after 30 minute (min) of incubation,<sup>44</sup> and (iii) finally, the EA addition yields again a negative  $V_{\rm D}$  shift of ~-0.1 V. The net  $V_{\rm D}$  change, just due to the preparation, is  $\sim 0.05$  V and sets a baseline for subsequent measurement related to Pb<sup>2+</sup> addition. The addition of EA serves for passivating any leftover PBASE molecules-which are not connected to the added Ap. Comparing the  $I_{\rm ds} vs~V_{\rm g}$ curve of the GFET after adding Ap-blue trace in Figure 2a, and after adding EA-green trace in Figure 2a, the latter indicates a higher slope (/transconductance) in the linear regime, then implying that the addition of EA could improve the sensor response, that is, a higher signal (electrical current:  $I_{\rm ds}$ ) is obtained for a given voltage  $(V_{\rm g})$  through the addition of the EA.

The electrical measurements were performed after ~10 min of incubation with various concentrations of Pb<sup>2+</sup> [from 1 fM upward (Figure 2b)] in aqueous electrolyte background prepared through mixing Pb(NO<sub>3</sub>)<sub>2</sub> with DI water. The saturation of the  $\Delta V_D$  with [Pb<sup>2+</sup>] > 1 nM (Figure 2c) indicates that the majority of the attached Ap has combined with the added Pb<sup>2+</sup>. Different [Pb<sup>2+</sup>] yield proportional amounts of the APG and could be correlated to the magnitude of the  $\Delta V_D$ . A continuous  $\Delta V_D$  shift to a more positive value



**Figure 3.** (a) Change in the  $V_D$  ( $\Delta V_D$ ) with [Pb<sup>2+</sup>], as a function of the Ap incubation time. A Hills–Langmuir model (with parameters listed in the *inset*) incorporating the influence of the Ap incubation times, that is, at 30 min: red, 2 h: yellow, and 4 h: blue, was used for modeling the associated Ap occupation and the binding kinetics. A LoD of ~61 fM, ~7 pM, and ~5 nM, were obtained, respectively. (b) Procedure for the determination of the limit of blank (LoB) and LoD for [Pb<sup>2+</sup>] with respect to the  $\Delta V_D$  for 30 min Ap incubation. (c) Variation of the transconductance ( $g_m$ ) with  $V_{gy}$  for different Ap incubation times. (d) Raman spectrum (normalized to maximum intensity) of the surface of *pristine* graphene compared to that on the *functionalized* graphene surface subsequent to 30 min, 2, and 4 h of Ap incubation. The shift of the 2D peak of graphene with increased Ap addition to higher wavenumber/s is indicative of *p*-type doping. The intensity of the *G*-band ( $I_G$ ) and *D*-band ( $I_D$ ) also increased with Ap incubation due to the doping (e). The ratio between 2D band intensity ( $I_{2D}$ ) and  $I_G$  steadily decreases with longer Ap incubation time.

with increasing  $[Pb^{2+}]$  was observed (Figure 2b,c). Such variation was understood in terms of increased *p*-doping of the graphene channel induced *via* the APG formation.<sup>14,15,39</sup> We quantify the extent of the related positive charge (*/hole*) induction, through a  $[Pb^{2+}]$  accrual induced carrier density  $(n_0)$ .<sup>45–47</sup> The  $n_0$  was parameterized through the product of a total capacitance ( $C_T$ ) and the $\Delta V_D$ , through

$$n_{0} = \frac{C_{T}(\Delta V_{\rm D})}{e}$$

$$\frac{1}{C_{\rm T}} = \left(\frac{1}{C_{\rm EDL} + C_{\rm P}} + \frac{1}{C_{\rm Q}}\right)$$
(1)

The  $C_{\rm T}$  was measured to be ~2  $\mu$ F/cm<sup>2</sup> (see Section S4 of the Supporting Information for detailed capacitance modeling and measurement/s) and constituted from a  $C_{\rm EDL}$ : an electrolyte double-layer capacitance in parallel to a capacitance  $C_{\rm p}^{48}$  contributed by the surface-immobilized Ap, along with a quantum capacitance ( $C_{\rm Q}$ ) in series with the  $C_{\rm EDL}$  and  $C_{\rm P}$ . The carrier (/hole) mobility ( $\mu$ ) in the graphene was then estimated from the following relation<sup>45–47</sup>

$$R_{\rm channel} = \frac{L/W}{e\mu n_{\rm tot}} = \frac{L/W}{e\mu \sqrt{\left(\frac{C_{\rm T}}{e}\right)^2 (V_{\rm g} - V_{\rm D})^2 + n_0^2}} = \frac{V_{\rm ds}}{I_{\rm ds}}$$
(2)

The  $R_{\text{channel}}$  is the resistance of the graphene channel, with L (= 500  $\mu$ m) and W (= 200  $\mu$ m) as the channel length and width. The  $(C_{\text{T}}/e)(V_{\text{g}} - V_{\text{D}}) = n_{\text{g}}$  is the gate-induced carrier density at different  $V_{\text{g}}$  with respect to the  $V_{\text{D}}$ , and  $n_{\text{tot}}$  (=  $\sqrt{n_0^2 + n_g^2}$ ) is a *net* carrier density.<sup>46</sup> The variation of the  $\Delta V_{\text{D}}$  with [Pb<sup>2+</sup>] is shown in Figure 2c, and the estimated  $\mu$  and  $n_0$  are shown in Figure 2d. It was observed that higher [Pb<sup>2+</sup>] leads to increasing  $n_0$  from ~2 × 10<sup>12</sup> to ~14 × 10<sup>12</sup> cm<sup>-2</sup> and an associated decreased  $\mu$  from ~740 to ~320 cm<sup>2</sup>/ Vs, arising from the additional scattering of the carriers on the graphene surface due to APG formation.

To further understand the observed response, we probed the binding affinity of APG to the graphene, deploying the Hills–Langmuir model.<sup>26</sup> Here, the  $\Delta V_{\rm D}$  is a function of the [Pb<sup>2+</sup>], through the relation (as explained in detail in Section S5 of the Supporting Information)

$$\Delta V_{\rm D} = A \frac{[{\rm Pb}^{2+}]^h}{K_d^h + [{\rm Pb}^{2+}]^h} + B$$
(3)

Here, A is the maximum  $\Delta V_{\rm D}$  response, presumably obtained when all Ap-binding sites are occupied. The B is a fit to the lowest expected  $\Delta V_{\rm D}$  as for a blank sample (GFET without [Pb<sup>2+</sup>]). It was observed that the A in eq 3 was increased with a longer incubation time, representing higher response, as also seen through an increased  $\Delta V_{\rm D}$  with higher

## Table 1. Determined LoD as a Function of the Ap Incubation Time

	$\overline{\Delta V_{\mathrm{D,B}}}$						
incubation time	(V)	$\sigma_{ m B}~({ m V})$	$\Delta V_{\mathrm{D,LoB}}$ (V)	LoB	$\sigma_{ m D}$ (V)	$\Delta V_{\mathrm{D,LoD}}$ (V)	LoD
30 min	0.030	0.0073	0.042	16.2 fM	0.0085	0.057	61.1 fM
2 h	0.022	0.0168	0.050	0.6 pM	0.0150	0.074	7.3 pM
4 h	0.063	0.0139	0.086	0.9 nM	0.0163	0.113	5.5 nM

 $[Pb^{2+}]$ : see *inset* to Figure 3a—due to a larger [APG] on the graphene surface.

The  $K_d$  is an equilibrium constant  $\left(=\frac{[APG]}{[Ap][Pb^{2+}]}\right)$  related to the APG formation, that is, with respect to the equilibrium

$$[Ap] + [Pb^{2+}] \rightleftharpoons [APG] \tag{4}$$

The square brackets indicate the respective concentrations. When the  $K_d$  equals the [Pb<sup>2+</sup>], the Ap on the graphene surface is half occupied by Pb<sup>2+</sup> (see Section S5 of the Supporting Information). The h is a coefficient related to the mutual interaction as manifested through a defined *cooperativity*.<sup>26</sup> The cooperativity is defined to be positive, that is, h > 1; if the binding of the Pb<sup>2+</sup> increases the receptor's (*i.e.*, Ap) apparent affinity and hence increases the chance of another Pb2+ binding, or negative, that is, h < 1; if the binding of one  $Pb^{2+}$  decreases the affinity of the Ap to another  $Pb^{2+}$ , as per the Hills–Langmuir mechanism. All our measurements of the h for the indicated incubation times was less than 1, for example, as seen in the inset to Figure 3a. The error bars were obtained through measurements on four devices tested at each concentration. The negative cooperativity arises from (i) the increasingly impeded binding of the Ap to the Pb<sup>2+</sup>, due to proximate APG formation, as well as (ii) increased Pb<sup>2+</sup> repulsion by induced positive charge carrier density from the APG.<sup>49</sup> For instance, with higher immobilized [Ap] on graphene, induced through longer incubation time, the  $n_0$ increases as more charges from the Ap are involved.

The previous considerations also gave high confidence that the Pb<sup>2+</sup> binding was on a one-to-one basis with the Ap, providing the extremely high and record selectivity as well as the specificity. It is noted from Figure 3a that the detection of Pb<sup>2+</sup> may be carried out over 13 orders of magnitude—from 10 mM to 1 fM. Here, different sensor preparation, through different incubation time/s were related to the relevant detection regimes, that is, 30 min for 1 fM to 100 nM, 2 h of incubation for 1 pM to 1  $\mu$ M, and 4 h of incubation for 1 nM to 1 mM region, respectively.

The plots of  $\Delta V_D vs \text{ Pb}^{2+}$  concentration (Figure 3a) indicate the necessity for considering the LoD and sensor response. A limit of blank (LoB) and an LoD, as a function of Ap incubation time, were estimated through the relations,<sup>50</sup> also see Figure 3b

$$\overline{\Delta V_{\text{D,B}}} + 1.645\sigma_{\text{B}} = \Delta V_{\text{D,LoB}} \leftrightarrow \text{LoB}$$
$$\Delta V_{\text{D,LoB}} + 1.645\sigma_{\text{D}} = \Delta V_{\text{D,LoD}} \leftrightarrow \text{LoD}$$
(5)

Here, the  $\overline{\Delta V_{\text{D,B}}}$  and  $\sigma_{\text{B}}$  represent the average and standard deviation of the  $\Delta V_{\text{D}}$  measurements of GFET on *blank* samples, that is, with no Pb<sup>2+</sup> analyte *and* only with deionized water. The aspect that 90% of the measurements would be accounted for is indicated through the  $1.645\sigma_{\text{B}}$  value correspondent to a standard normal distribution.<sup>50</sup> For example, with 30 min Ap incubation, we obtained from the

fits in Figure 3a that  $\overline{\Delta V_{\text{D,B}}} = 0.030 \text{ V}$  and  $\sigma_{\text{B}}$  of 0.0073 V. The determined LoB is 16.2 fM—corresponding to the  $\Delta V_{\text{D,LoB}} = 0.042 \text{ V}$ . Subsequently, the LoD was determined from the  $\sigma_{\text{D}}$  (= 0.0085 V) per the standard deviation of responses relative to the lowest [Pb<sup>2+</sup>] detectable (~1 fM). With an estimated  $\Delta V_{\text{D,LoD}}$  of 0.057 V, the LoD was estimated at 61 fM.

Similarly, the LoB and the LoD were determined for the measurements on the GFET with 2 and 4 h of Ap incubation time (Table 1) and correlated to a  $K_d$  of 10.5 nM and 1.0  $\mu$ M, respectively. The change in the *immobilized* [Ap]—through the  $K_d$ —from pM to  $\mu$ M, indicates an effective increase in the interaction of the Ap with the PBASE on graphene, presumably due to diffusional limitations. Furthermore, the *immobilized* [Ap] seems limited by the incubation time rather than the *bulk* [Ap], see Section S6 in the Supporting Information.

A higher LoD with longer Ap incubation time, that is, larger *immobilized* [Ap], could be attributed to a decrease in  $\mu$  and correlated to the FET device transconductance:<sup>51</sup> $g_{\rm m} = \partial I_{\rm ds}/\partial V_{\rm g}$  (at a given  $V_{\rm ds}$ ). For example, with 30 min, 2, and 4 h incubation at different  $V_{\rm g}$ : Figure 3c, we observed a higher  $g_{\rm m}$  (with a maximum ~130  $\mu$ S) in the case of 30 min, while the maximum  $g_{\rm m}$  reduces for 2 and 4 h incubation (with a maximum ~90 and ~50  $\mu$ S, respectively). The  $\mu$  obtained through eq 2 yielded a lower (/higher) value of 514 (/820) cm<sup>2</sup>/Vs with Ap incubation time of 4 (/0.5) h, respectively. The decrease in  $\mu$  may be associated with an increased  $n_0$  at higher *immobilized* [Ap]. Since maximal sensing response may be reached when GFET is operated at maximal  $g_{\rm m}$ , <sup>42</sup> a higher  $\mu$  with a higher  $g_{\rm m}$  would be critical in optimizing the LoD.

Further investigation of carrier doping was carried out through Raman spectroscopy (at 532 nm, Renishaw inVia) on the pristine graphene and then on graphene with the immobilized Ap at different incubation times (30 min, 2, and 4 h) (Figure 3d). In all cases, the D (at  $\sim$ 1350 cm<sup>-1</sup>) and D' (as a higher wavenumber shoulder to the G-peak at  $\sim 1520$ cm<sup>-1</sup>) peaks were obtained—the intensity of which increased with increasing incubation time. Such correlation of the D and D' peak intensity with PBASE addition was indicated<sup>35</sup> as validating the  $\pi-\pi$  stacking of PBASE onto the graphene surface and related non-covalent functionalization. We have also noted the influence of incubation time on the sensing surface through detailed Raman spectroscopy, where it was found that the obtained spectra were reliable at only after  $\sim 30$ min of incubation time. It was also observed that the 2D peak of graphene with the Ap shifted to higher wavenumber (from  $\sim 2660$  to  $\sim 2667$  cm<sup>-1</sup>), indicative of hole doping.<sup>52,53</sup> The intensity of the G band  $(I_G)$  and D band  $(I_D)$  also increased with Ap incubation as related to an increased  $n_0$ .<sup>54,55</sup> The ratio between 2D band intensity  $(I_{2D})$  and  $I_G$  decreases from ~2.9 to  $\sim$ 1.5 with the addition of the Ap and longer incubation time (Figure 3e).

The discriminative detection of  $Pb^{2+}$  in aqueous solutions, over other commonly found ions, for example,  $Ca^{2+}$ ,  $Co^{2+}$ ,  $Al^{3+}$ ,  $Fe^{3+}$ , and so forth, is indicated in Figure 4. Generally, a



**Figure 4.** Specificity for Pb<sup>2+</sup> detection *over* alternate ions, that is, Ca<sup>2+</sup>, Co<sup>2+</sup>, Fe<sup>3+</sup>, and Al<sup>3+</sup>, that may be present in aqueous solutionbased analytes, indicated through the respective  $\Delta V_{\rm D}$  over an ion concentration range of 1 fM to 100 nM. The upper and lower bond of the error bar indicates standard deviation of the measured  $\Delta V_{\rm D}$  from different GFET devices. A threshold value for the  $\Delta V_{\rm D,T}$  of 0.05 V can be used to specifically identify Pb<sup>2+</sup> when the electrolyte concentration  $\geq 1$  pM.

modulation of the Ap conformation could arise due to both the  $Pb^{2+}$  and the alternate ions. While the  $Pb^{2+}$  binds to the Ap, the other ions perturb the Ap to a smaller extent. The latter aspect is ensured through the careful selection of the Ap on the basis of its specific affinity<sup>37-40</sup> with Pb<sup>2+</sup>, as indicated previously, that is, through using a ssDNA-based Ap with multiple G in the sequence to enhance the Ap-Pb<sup>2+</sup>-binding affinity, with low affinity to other ions. It is consequently expected that other ions would not be able to form a related G-quadruplex.<sup>37-40</sup> To verify such assumption,  $Ca^{2+}$ ,  $Co^{2+}$ ,  $Al^{3+}$ , and  $Fe^{3+}$ electrolyte solutions were prepared through dissolving associated nitrate salts into DI water, with dilution over a range of concentrations. An Ap  $(10 \,\mu\text{M})$  incubation time of 30 min, was chosen, given the better LoD, as indicated previously. The statistics from the tests in aqueous media on different GFET devices, along with the obtained  $\Delta V_{\rm D}$ , are reported in Figure 4.

While a pronounced positive  $\Delta V_{\rm D}$  was observed for Pb<sup>2+</sup>, in the case of the other ions, the  $\Delta V_{\rm D}$  was indifferent and close to zero, that is, there was no significant modulation with respect to the baseline  $\Delta V_{\rm D}$ , measured in DI water (Figure 4). The difference between the binding of the Pb<sup>2+</sup>, and the perturbation from the other ions, was addressed by introducing a threshold voltage  $\Delta V_{\rm D,T}$  (= 0.05 V, *red dashed* line), which *distinctly* indicates the presence of Pb<sup>2+</sup> ion, at a concentration >10<sup>-12</sup> M, that is, when the measured  $\Delta V_{\rm D} > \Delta V_{\rm D,T}$ . Consequently, the data in Figure 4, demonstrate the specificity of Pb<sup>2+</sup> ion, at the 1 pM level, through such a defined threshold.

## CONCLUSIONS

Our studies indicate a superior LoD-related sensitivity of ~61 fM, along with specificity at the 1 pM level, *both* of which are significantly better, by orders of magnitude, compared to previous work on Ap-based Pb<sup>2+</sup> detection, as detailed in Table 2. We have shown, through extensive characterization and consequent developed understanding, that key factors for the *record* specificity and LoD incorporate: (1) the selection of the Ap, along with (2) the optimization of the immobilized Ap

Table 2. Comparison with the Published Sensitivity and the Specificity of Ap-Based Pb<sup>2+</sup> Sensors

methods and materials	sensitivity (LoD)	specificity	Ap incubation
Au electrode; APG <sup>56</sup>	35 nM	$1 \ \mu M$	120 min; 4 μM
Au electrode + Au nanoparticles (NPs); APG <sup>57</sup>	4 pM	70 nM	60 min; 15 μM
GFET, APG + methylene blue <sup>15</sup>	2 µM	-not reported-	2 h;15 µM
GFET, APG <sup>14</sup>	790 pM	48 nM	4 h;1 µM
GFET, pyrene-derivatized 8–17 DNAzyme <sup>16</sup>	181 pM	0.5 nM	30 min; 4 μM
GFET + Au NPs; pyrene-derivatized 8–17 DNAzyme <sup>17</sup>	20pM	0.1 nM	2 h;5 µM
GFET, electrolyte gated, APG	61 fM	1 pM	30 min; 10 μM

surface occupation density as a function of the incubation time. Such aspects have not been brought to the fore in previous studies and help interpret earlier measurements<sup>56,57</sup> as well. Our careful studies and quantification lay the basis for the next-generation  $Pb^{2+}$  detection sensors with the advantages of scalable fabrication, low-cost, along with high sensitivity, and specificity. Future work would focus on the use and testing of the developed sensor in a variety of practical environments with comparison to detection of lead ion concentration with other methods.

## METHODS

Graphene Synthesis. Single-layer graphene (SLG) was synthesized through low pressure (300 mTorr) chemical vapor deposition (LPCVD) on Cu foil (25  $\mu$ m thickness, MTI Corp), through using a mixture of hydrogen ( $H_2$ , 15 sccm) and methane ( $CH_4$ , 2 sccm) for 2 h. The foil was pre-cleaned by trichloroethylene followed by acetone and isopropyl alcohol (IPA) rinsing and N<sub>2</sub> blow dry. The pre-cleaned Cu foil was folded to ensure stable precursor flow,<sup>58</sup> resulting in highquality large area SLG growth. The Cu foil was annealed for 1 h at 1000 °C, under 85 mTorr, to reduce residual contamination. The LPCVD-grown SLG on Cu was spin-coated (at 3000 rpm for 45 s) by 120 K MW poly (methyl methacrylate) (PMMA) for subsequent wet transfer<sup>59</sup> onto an electrode-patterned SiO<sub>2</sub>/Si substrate. Oxygen  $(O_2)$  plasma etching was used to remove the SLG on the backside of the Cu foil. Ammonium persulfate (0.1 M) solution was used to etch Cu foil and subsequently rinsed with DI water. The PMMA was dissolved using acetone for 1 h followed by IPA rinse and N<sub>2</sub> blow dry. The GFET fabrication was conducted through thin-film deposition, photolithography, and plasma etching, as described in Figure S2 of the Supporting Information.

**Device Measurement.** The GFET  $I_{ds}-V_g$  measurement was performed using two source meters (Keithley 2400), one for applying constant source drain voltage  $V_{ds}(= 0.2 \text{ V})$  and monitoring the  $I_{ds'}$  the other for supplying  $V_g$  and monitoring the gate current ( $I_g$ ). Prior to an  $I_{ds}-V_g$  measurement, the GFET sensor was pre-cleaned by DI water three times after the incubation of PBASE, Ap, and EA. The varying [Pb<sup>2+</sup>] is directly measured in an electrolyte, with varying [Pb<sup>2+</sup>]. The  $V_g$  was swept from negative voltage to positive voltage and backward for three cycles, at ~30 mV/s. The Dirac voltage ( $V_D$ ) was estimated from the  $V_g$  corresponding to the  $I_{ds}$  minimum in each cycle. For all measurements, the gate leakage current was found to be less than ~10 nA.

#### ASSOCIATED CONTENT

## **Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsanm.2c05542.

On graphene characterization, field-effect transistor fabrication, attachment of amine-functionalized Ap to PBASE, determination of the constituent capacitance contributions from the  $Pb^{2+}$  complexed graphene FET, binding equilibria of the APG, and the influence of varying Ap concentrations (PDF)

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### **Author Contributions**

Y.D., D.K.B, and P.R.B., contributed to the conceptualization, experimental design, and the development of the project. Y.D. carried out the electrochemical measurements and Raman spectroscopy. A.L. and K.W. worked on the modeling and interpretation of the results. Y.D. and P.R.B. wrote the paper. All the authors analyzed and discussed the results.

### Notes

The authors declare no competing financial interest.

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