Electronic Supporting Information for the Article:

A Graphene Oxide-based Sensing Platform for Lead (II) Ions Analysis with Tunable Dynamic Range by using Pb^{2+}-dependant DNAzyme

Yanqin Wen,† Cheng Peng,† Di Li,†* Lin Zhuo,‡ Shijiang He,† Lihua Wang,† Qing Huang,† Qing-hua Xu,‡ Chunhai Fan†*

†Laboratory of Physical Biology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800 (China)
‡ Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore

Experimental Section

Materials

The DNA/RNA oligonucleotides were synthesized and purified by TaKaRa Biotechnology Co. (Dalian, China). The following oligonucleotide sequences were used:

17S: 5'- Cy3-ACTCACTAT*rA*GGAAGAGATGTCTGT-3' (The bold italic letter is the cleavage site).

17E: 5'-ACAGACATCTCT TCTCCG AGCCGGTCGAAATAGTGAGT-3'

$17E_C$: 5'-ACAGACATCTCTTCCCCGAGCCGGTCGAAATAGTGA GT-3'

$Pb(NO_3)_2$ was purchased from Aldrich and used as received. $Cu(NO_3)_2$, $Zn(NO_3)_2$, $Cd(NO_3)_2$, $Co(NO_3)_2$, $Mn(CH_3COO)_2$, $Ni(NO_3)_2$ and $Hg(NO_3)_2$ were of analytical grade and used as received. All solutions were prepared with Milli-Q water (18 MΩ cm^{-1}) from a Millipore system. Environmental water sample was taken from a nearby river and centrifuged to remove the insoluble impurities.

Preparation of GO

GO was synthesized from graphite powder based on the Hummer's method. Briefly, graphite power (4 g) was oxidized in a hot solution (80°C) of concentrated H_2SO_4 (24 mL) containing $K_2S_2O_8$ (8 g), and P_2O_5 (8 g). The resulting dark blue mixture was

thermally isolated and slowly cooled to room temperature over a period of 6 h. The mixture was diluted to 300 mL, and then filtrated with a filter membrane of 0.22 μm (Generay Biotech Co., Ltd., Shanghai, China) and dried overnight at 60°C. These preoxidized graphite powder (2 g) was added to 92 mL of cold H_2SO_4 (0°C), to which $KMnO_4$ (12 g) was gradually added under continuous stirring in ice-bath. After 15 min, $NaNO_3$ (2 g) was added to the mixture. The solution was further stirred for 2 h at 35 °C and distilled water (200 mL) was added. The reaction was stopped with the addition of a mixture of 560 mL of distilled water and 10 mL of H_2O_2 (30 %). The product was washed with HCl (1:10) and then with water, and then suspended in distilled water. The brown dispersion was extensively dialyzed to remove residual metal ions and acids, and then exfoliated via sonication for 1.5 h (300 W). Unexfoliated graphite oxide was removed by centrifugation (3000 rpm, 5 min) using Centrifuge himac-CF 16RX (Hitachi, Japan).

Fluorescence assay for Pb(II) Ions

Pb (II) ions of different concentrations were incubated in Tris-HCl buffer (50 mM, pH 7.4) containing 50 mM of NaCl and 20 nM of DNAzyme for 30 min at room temperature. Then 4 μL of GO (2.5 μg/μL) was added to this mixture and the fluorescence measurement was carried out 1 min after the GO addition.

Instruments

The fluorescence spectra were measured using a Hitachi F-4500 spectrophotometer equipped with a Xenon lamp excitation source. The excitation wavelength was λ=545 nm, and the fluorescence measurements were carried out at 23^{o}C. The time-resolved fluorescence was measured by using the time-correlated single photon counting (TCSPC) technique. The experiments were conducted on a Fluorolog HORIBA JOBNYVON instrument with instrument response function (IRF) of 260 ps. A HORIBA JobinYvon pulsed laser diode at 488 nm was used as the excitation source for all samples.

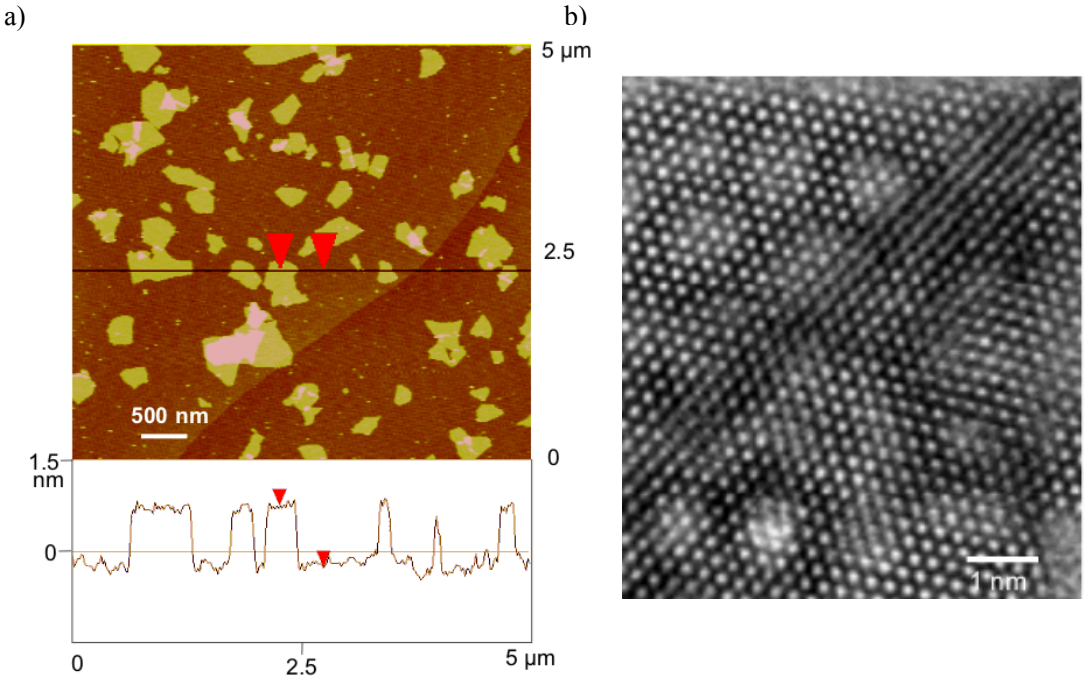

Figure S1. a) AFM tapping-mode image of the as-prepared GO sheets and the height profile along the dashed line in panel. b) HR-TEM image of as-prepared GO.

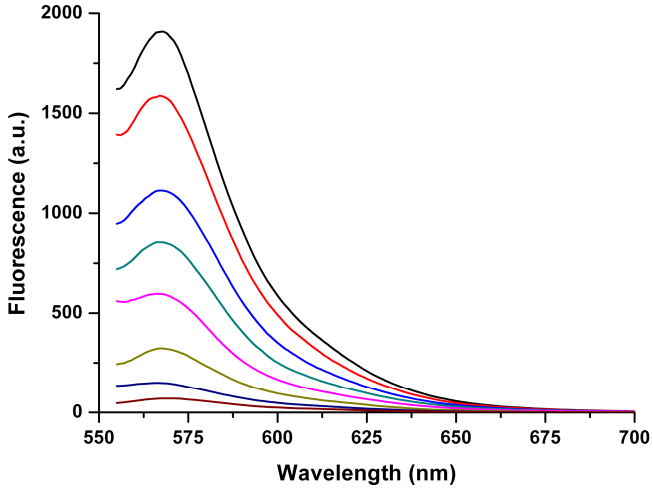

Figure S2. Fluorescence quenching of Cy3 (30 nM) in the absence (black) and presence of a series of amounts of GO (top to bottom: 0.25, 0.50, 1.00, 1.25, 1.50, 1.75 and 2.00 µg).

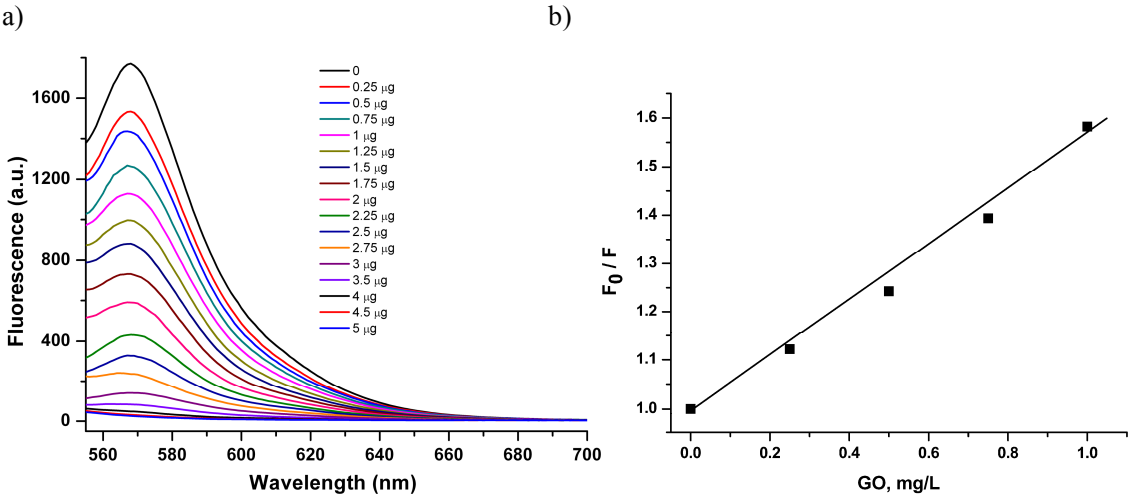

Figure S3. a) The fluorescence spectra of Cy3-tagged 17S upon incubation with different amounts of GO. b) The Stern–Volmer plots of the Cy3-labeled 17S quenched by GO. The K_{sv} was calculated as 0.63 L mg^{-1} for Cy3.

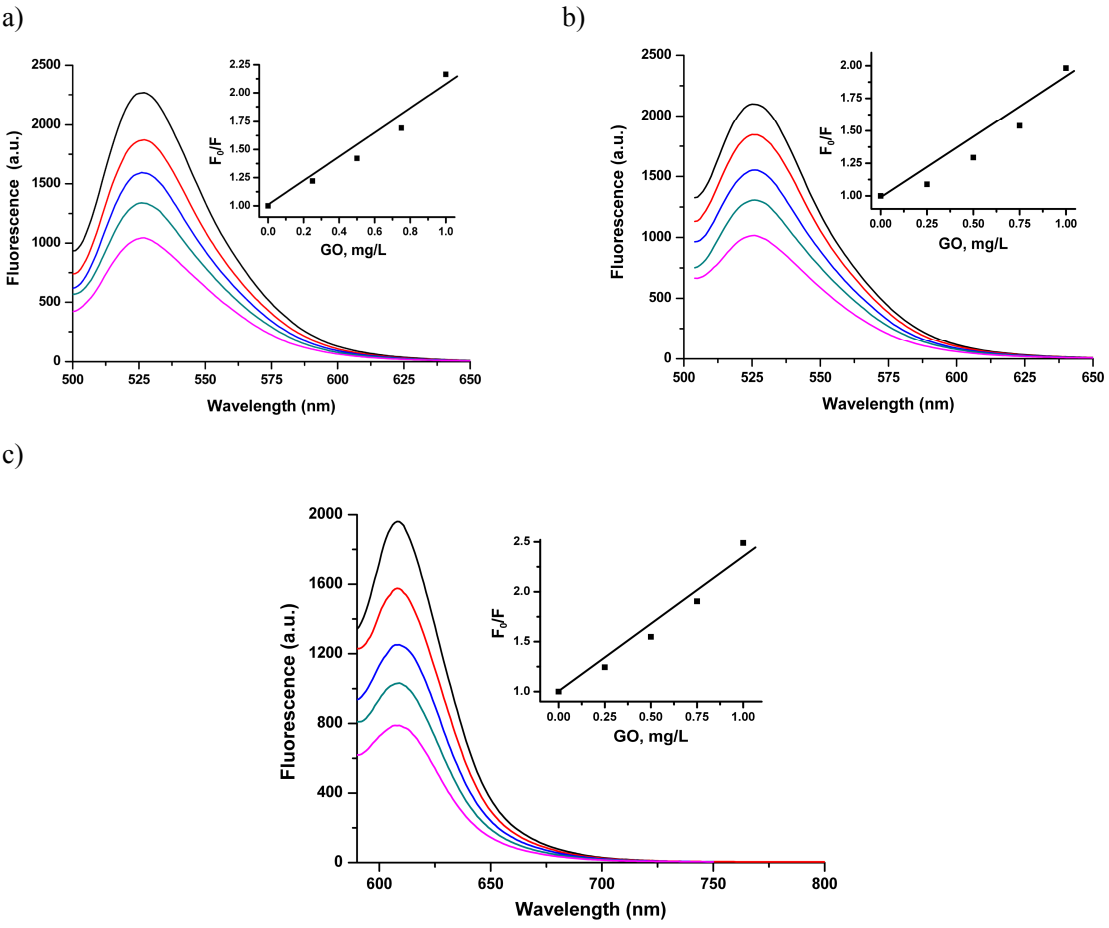

Figure S4. The GO concentration-dependent fluorescence quenching of FITC (a), FAM (b) and ROX (c)-labeled ss-DNA (all of 20 nM). Insets are the Stern–Volmer

plots of these dye-labeled SS-DNA quenched by GO. The K_{sv} was calculated as 1.12 L mg^{-1}, 0.97 L mg^{-1} and 1.46 L mg^{-1} for FITC, FAM and ROX, respectively.

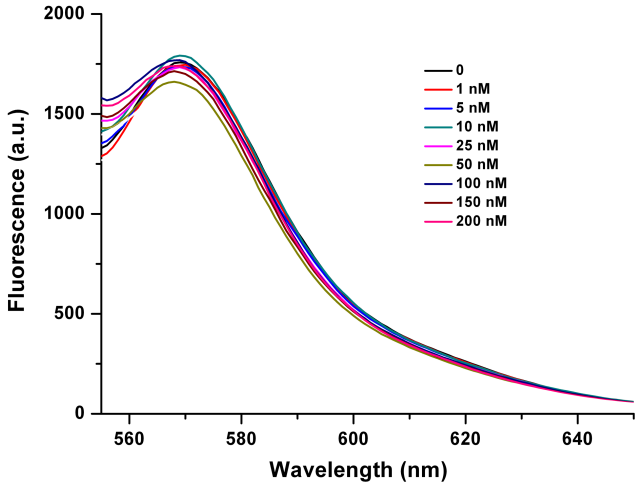

Figure S5. Fluorescence spectra of the Cy3-labeled 17S strand upon incubation with different concentrations of Pb^{2+}.

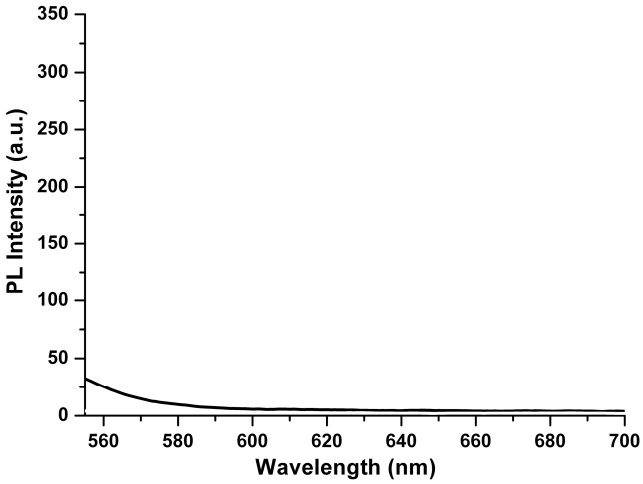

Figure S6. Photoluminescence spectrum of GO. λ_{ex} = 545 nm

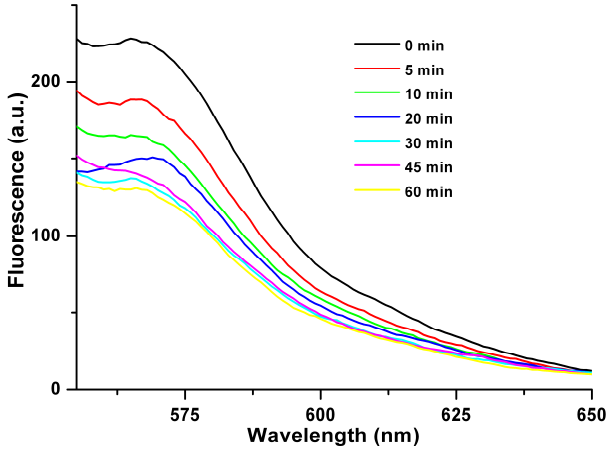

Figure S7. Fluorescence spectra of DNAzyme upon incubation with 10 nM Pb^{2+} for different time intervals at room temperature and then mixed with 10 μg GO. All other experimental conditions are identical to those in Scheme 1.

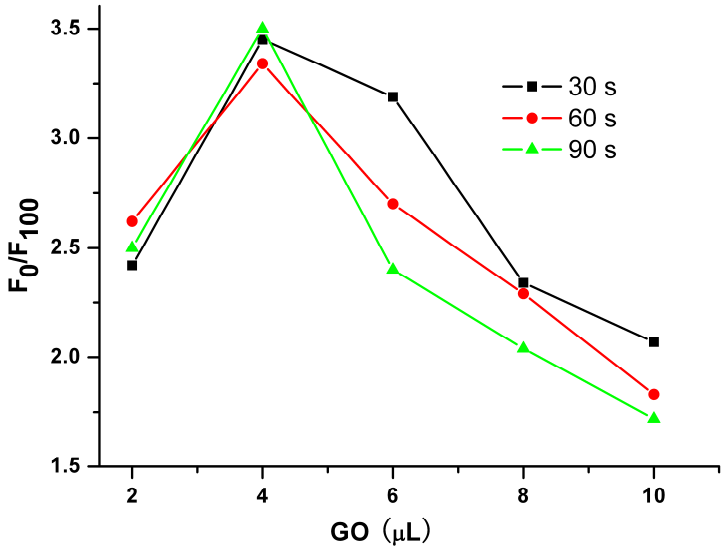

Figure S8. Optimization of the amount of GO addition. 100 nM of Pb^{2+} of lead (II) was incubated with DNAzyme for 30 min, then different volume of GO (2.5μg/μL) was added to the mixture. The fluorescence spectra were recorded after 30, 60 or 90s of the addition. The y-axis of this figure of the ratio of fluorescence intensity of background signal (F_o) to that of 100 nM of Pb^{2+} (F_{100}).

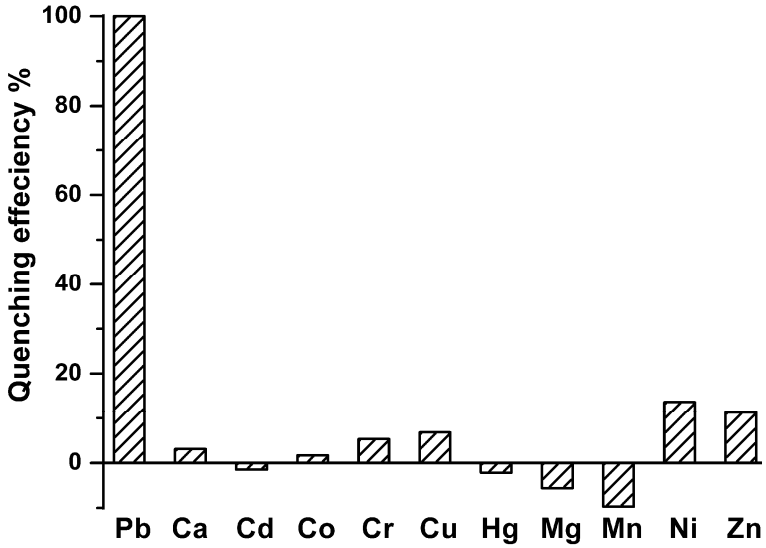

Figure S9. Selectivity of the present assay towards Pb^{2+} ions over other 10 interference ions. The concentration of all the metal ions was 1 μM.

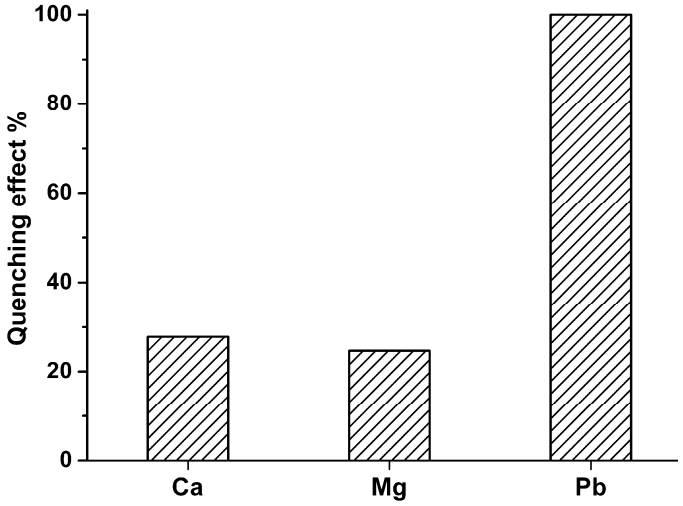

Figure S10. Selectivity of the present assay towards Pb^{2+} ions (1 μM)over Ca^{2+} and Mg^{2+} of higher concentrations (1 mM) since the concentration of Ca^{2+} and Mg^{2+} in real environmental samples are at mM range.

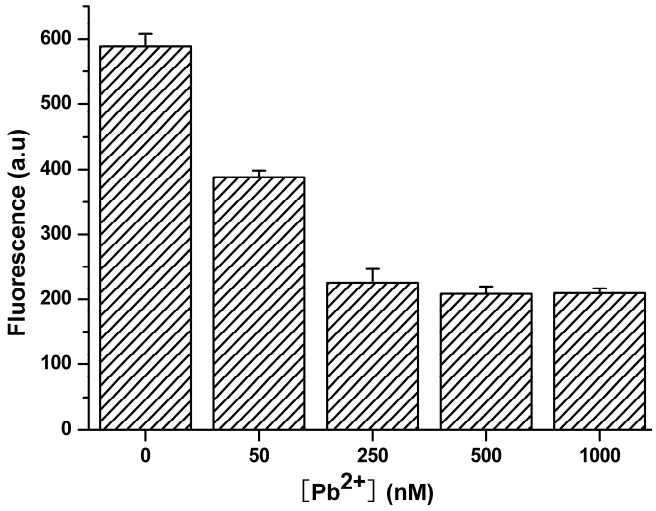

Figure S11. Comparison of fluorescence responses of this GO-based sensor for different concentrations of Pb^{2+} dissolved in real environmental water samples.