Electronic Supplementary Information

A novel electrochemical DNAzyme sensor for the amplified detection of Pb2+ ions

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Reagents and chemicals

Oligonucleotides were synthesized and purified by Sangon Inc. (Shanghai, China). The sequences of these oligomers are shown below:

DNA1: 5'-HS-$(CH_2)_6$-TTT TTC ATC TCT TCT CCG AGC CGG TCG AAA TAG TGA GT-3'

DNA2: 5'-ACT CAC TAT rAGG AAG AGA TG-3'

DNA3: 5'-ACT CAC TAT TTC-$(CH_2)_6$-HS-3'

Here, DNA1 is the Pb^{2+}-specific DNAzyme, DNA2 is the substrate oligonucleotide. Tris(2-carboxyethyl)phosphinehydrochloride (TCEP), hexaaminerutheium(III) chloride ($[Ru(NH_3)_6]Cl_3$), tris(hydroxymethyl)aminomethane, 6-mercaptohexanol (MCH), and $HAuCl_4 \cdot 3H_2O$ were purchased from J&k Chemical Ltd. All other chemicals were of analytical grade. All solutions were prepared with ultrapure water (18.2 MΩcm) from a Millipore Milli-Q system (Sartorius, Germany).

Synthesis of double stranded functionalized gold nanoparticles

The 13± 2.2 nm AuNPs determined by transmission electron microscope (JEOL, Japan) were prepared using a usual citrate reduction method[1-2] with some modification. In brief, a 100 mL aqueous solution of 0.01% (w/v) $HAuCl_4$ was added into a round-bottom flask, stirred to boil. Then 3.5 mL 1% trisodium citrate was added rapidly into the boiling solution. The color of the solution became from colorless to wine red after boiling for another 15 min with stirring. After stirring for another 15 min, the solution was cooled down to room temperature. The concentration (2.2 nM) was calculated from the quantity of starting material ($HAuCl_4$) and the size of AuNPs at the wavelength of 519 nm.[3]

The functionalization of the prepared AuNPs with DNA1 was achieved by the well-known gold-thiol chemistry,[4] which was also beneficial to the stability of AuNPs.[5] Briefly, AuNPs solution (2.2 nM) was centrifuged at 12,000 rpm for 20 min, and then 80% supernatant was removed. Then the residual AuNPs solution was mixed with DNA1 with the final concentration of 3.0 μM DNA1 and 10 nM AuNPs under room temperature for 24 h in darkness. Then the DNA1-AuNPs conjugates were incubated in salts (0.3 M NaCl, 25 mM

tris-acetate, pH 8.0) containing 15 μM DNA2 to make DNA1 hybridize with DNA2 in a 65 °C water bath for 10 min. The hybridization and "aging" lasted another 24 h at room temperature to obtain ordered dsDNA on the surfaces of AuNPs. The excess regents were removed by centrifuging twice at 12, 000 rpm for 20 min at 4 °C. The precipitate was washed, recentrifuged, and then dispersed in 25 mM tris-acetate buffer (pH 8.0). The solution was stored at 4 °C when not in use.

Preparation of DNA3 modified gold electrodes

The gold electrode used for the self-assembled monolayer (SAM) was first cleaned using the usual procedure.[6] In brief, the bare gold electrode was first polished in the aqueous slurry of alumina and rinsed with deionized water. Residual alumina particles were thoroughly removed by sonicating electrodes in ethanol and deionized water for 5 min, respectively. Then the electrode was etched for about 5 min in a '*Piranha*' solution (98% H_2SO_4:30% H_2O_2 = 3:1(v/v)) and then rinsed with copious amounts of water followed by ethanol to remove organic contaminants (*Caution! Piranha solution reacts violently with organic materials and should be handled with extreme care!*). Finally, the electrode surface was established by incubating it in 0.5 M H_2SO_4 and scanning the potential between 0 and 1.6 V at the scan rate of 50 mV s^{-1} until a reproducible scan was obtained.

After being dried with nitrogen, the cleaned gold surface was incubated in a solution composed of 1 μM DNA3, 25 mM tris-acetate buffer (pH 7.4), and 1 mM TCEP (which is included to reduce disulfide bonded oligomers) for 10 h. The surface was then passivated with 1 mM MCH for 2 h to reduce nonspecific adsorption of DNA and to obtain a well aligned DNA monolayer.[7] MCH could not replace oligonucleotides on the surface in thermodynamics.[8]

Assembly of DNA-AuNPs on electrode surface

Firstly, the dsDNA-AuNPs reacted with various concentrations of Pb^{2+} in buffers (25 mM tris-acetate, 0.3 M NaCl, pH 8.0) for 1 h. Then the resulting products were immobilized onto the DNA3 modified electrode by immersing the electrode in the solution for another 12 h. Finally, the electrode was immersed in buffer (10 mM tris-acetate, pH 8.0) for 10 min to reduce the nonspecific adsorption of DNA-AuNPs.

Electrochemical measurements and calculations

Electrochemical measurements were carried out at room temperature with an electrochemical workstation (CHI 660C, CH Instruments). A conventional three-electrode cell, consisting of a modified gold working electrode, a large area platinum sheet auxiliary electrode, and a

saturated reference calomel electrode (SCE), was used for the electrochemical measurement. All potentials are referred to the SCE. 10 mM tris-acetate buffer (pH 8.0) was used as the electrolyte. $[Ru(NH_3)_6]^{3+}$ molecules were used as electrochemical probes for cyclic voltammetry (CV) and chronocoulometry (CC) experiments. The scan rate was 50 mV s^{-1} for CV, and the pulse period was 0.25 s for CC. Buffers were purged with highly purified nitrogen for at least 15 min prior to a series of electrochemical experiments. A nitrogen environment was kept in the cell during the whole experiment. For each concentration of Pb^{2+}, the measurement was repeated for at least three times. The limit of detection (LOD) can be obtained by the equation *LOD = 3 * standard deviation of the blank*, where *standard deviation of the blank* was obtained by measuring 7 blank samples.

References

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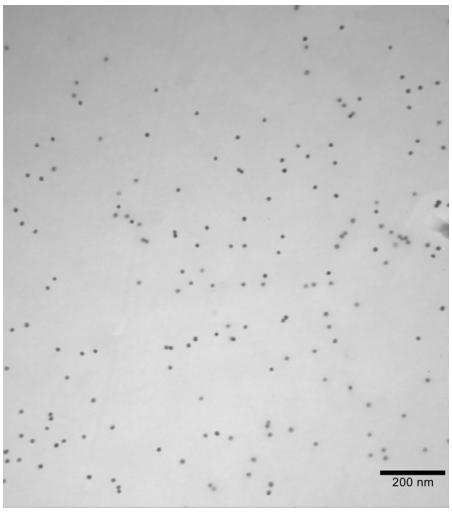

Fig. S1 TEM image of the 13± 2.2 nm AuNPs

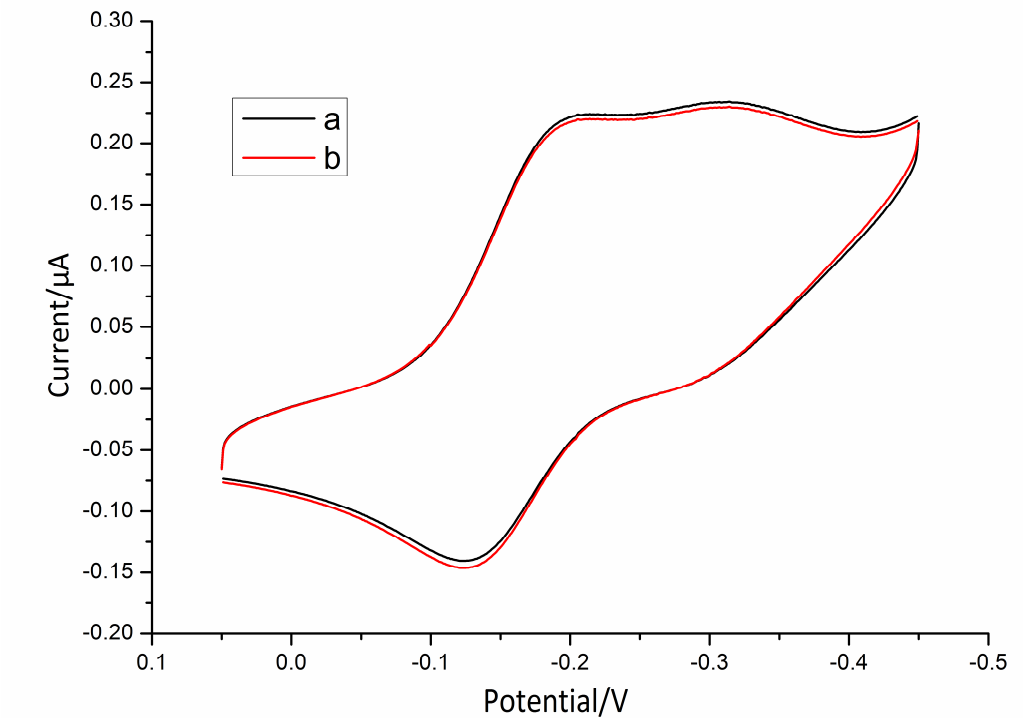

Fig. S2 Cyclic voltammograms of 50 μM $[Ru(NH_3)_6]^{3+}$ obtained at the DNA3 modified gold electrodes after incubating the electrode in (a) buffer; (b) dsDNA-AuNPs solution. (Both in the absence of Pb^{2+})

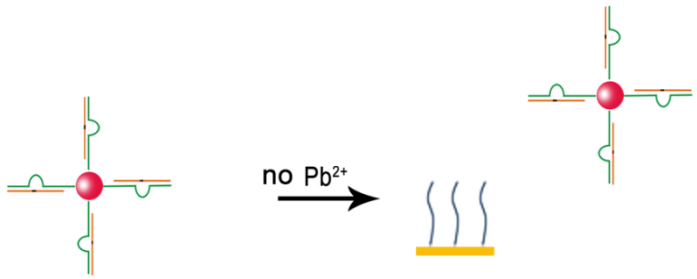

Fig. S3 The double stranded DNA functionalized AuNPs can barely be immobilized onto the electrode surface in the absence of Pb^{2+} due to the thorough hybridization of DNA1 with DNA2. The background response is ascribed exclusively to the short stranded DNA3 (12-base) which can only adsorb a small quantity of RuHex, so the background level is very low.

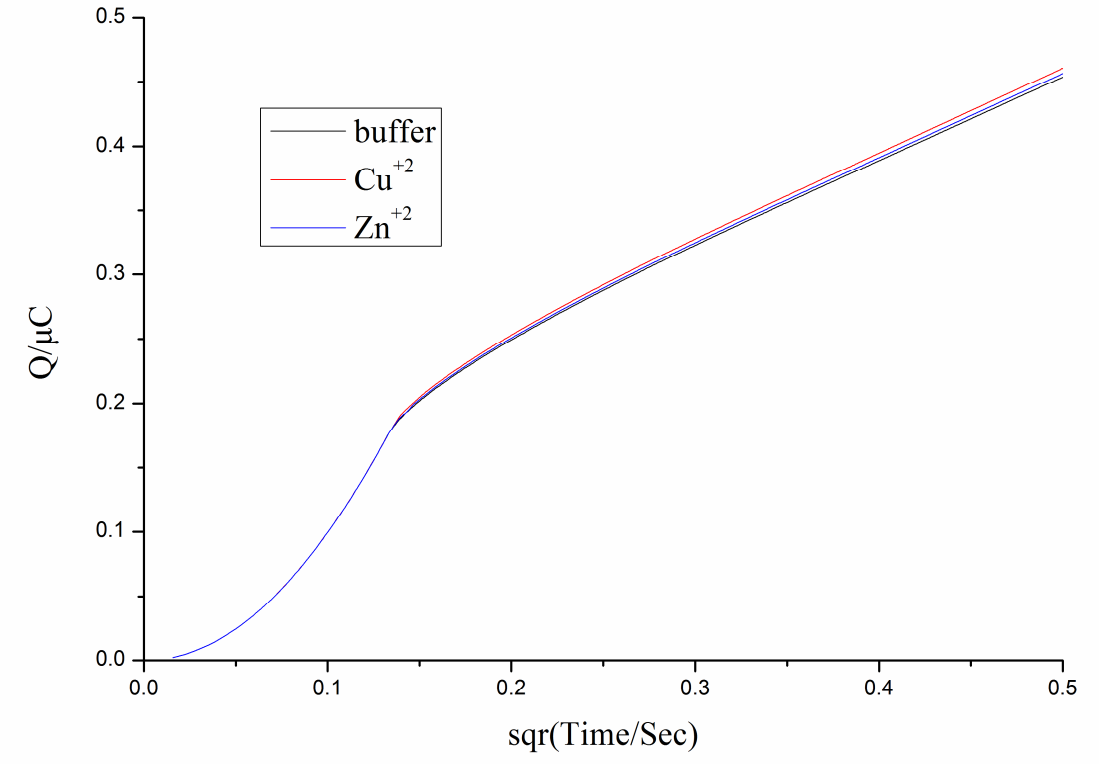

Fig. S4 The sensor does not exhibit any significant signal change when challenged with 40 nM Cu^{2+} or Zn^{2+}. The electrochemical signals arise from the buffer (10mM tris- acetate, pH 8.0) and from the same buffer doped with 10 mM Cu^{2+} or Zn^{2+}.

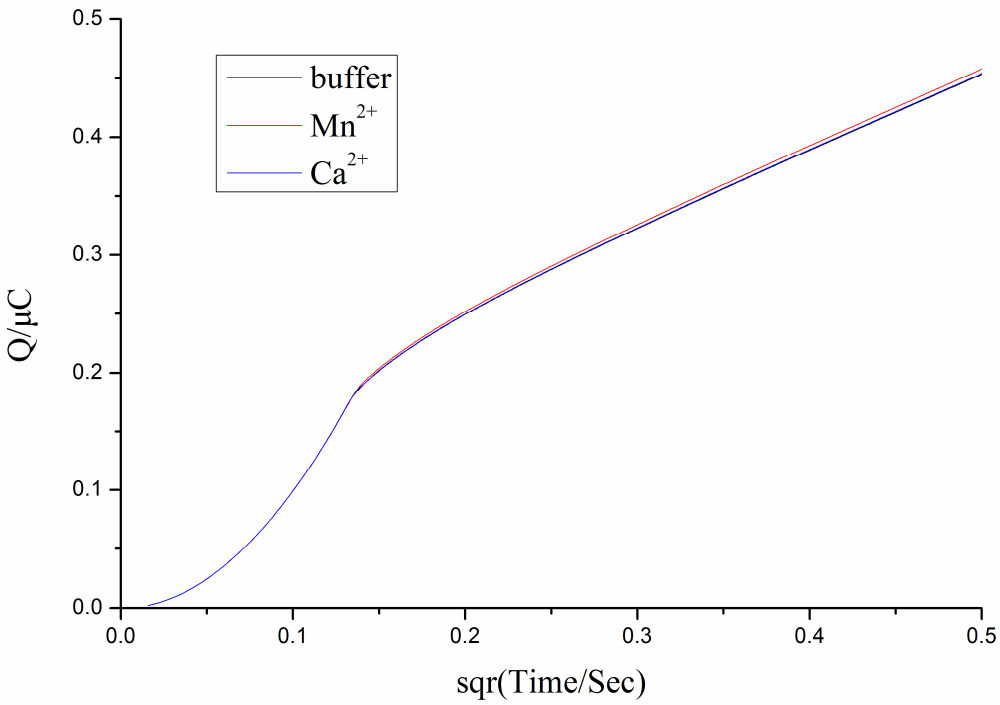

Fig. S5 The sensor does not exhibit any significant signal change when challenged with 40 nM Mn^{2+} or Ca^{2+}. The electrochemical signals arise from the buffer (10mM tris- acetate, pH 8.0) and from the same buffer doped with 10 mM Mn^{2+} or Ca^{2+}.

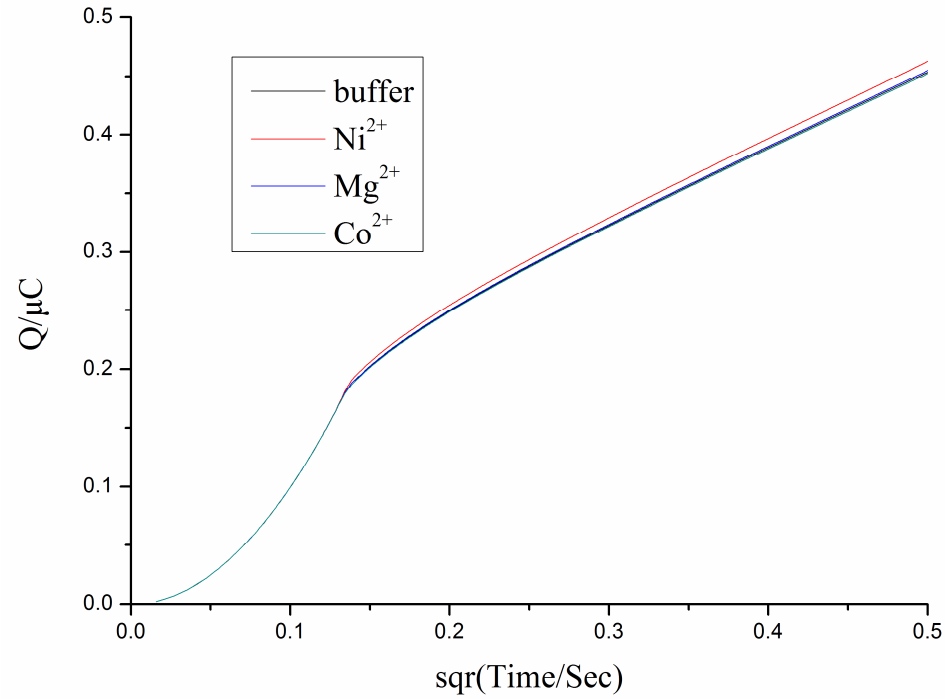

Fig. S6 The sensor does not exhibit any significant signal change when challenged with 40 nM Ni^{2+}, Mg^{2+} or Co^{2+}. The electrochemical signals arise from the buffer (10mM tris- acetate, pH 8.0) and from the same buffer doped with 10 mM Ni^{2+}, Mg^{2+} or Co^{2+}.