Electronic Supplementary Information

In Situ Electro-polymerization of Nitrogen doped Carbon Dots and Its Application in Electrochemiluminescence Biosensor for the Detection of Intracellular Lead Ion

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Experimental

Reagents and material

Potassium persulfate ($K_2S_2O_8$) and ascorbic acid (AA) were purchased from Kelong Chemical Company (Chengdu, China). Potassium tetrachloropalladate (K_2PdCl_4) and gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$) were obtained from Kangda Company (Shanghai, China). Silver nitrate ($AgNO_3$), o-phenylenediamine (OPD), lead nitrate ($Pb(NO_3)_2$), trisodium citrate dihydrateand ($Na_3C_6H_5O_7 \cdot 2H_2O$), cetylpyridinium chloride monohydrate (CPC), hexanethiol (HT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Phosphate buffer solutions (PBS) with pH 7.4 were prepared by mixing standard stock solutions of 0.1 M K_2HPO_4, 0.1 M NaH_2PO_4, and 0.1 M KCl and adjusting the pH with 0.1 M HCl or NaOH, then diluting with ultrapure water. All chemicals were of analytical grade and used without further purification. Hela cells were provided by the cell bank of the Committee on Type Culture Collection of Chinese Academy of Science (Shanghai, China). All

solution was prepared with ultrapure water and stored in the refrigerator (4 °C). The DNA probes were synthesized by Sangon, Inc. (Shanghai, China). The nucleotide sequences are listed in Table S1.

Table S1 Sequence information for the nucleic acids used in this study

name	sequences*(5′-3′)
capture DNA (T_1)	NH_2-ATC GAA AAT
complementary DNA (T_2)	GGG GAT TTT CGA T-NH_2
ssDNA1 (S_1)	GGG TGG GTG GGT GGG TAG AAT TGT ACT TAA ACA CCT T-NH_2
ssDNA2 (S_2)	GGG TGG GTG GGT GGG TAA GGT GTT TAA GTA CAA TTC T-NH_2

Apparatus

The ECL intensity was monitored by a model MPI-A electrochemiluminescence analyzer (Xi'An Remax Electronic Science & Technology Co. Ltd., Xi'An, China). The voltage of the photomultiplier tube (PMT) was set at 800 V. The ECL spectrum was obtained from a model MPI-E II electrochemiluminescence analyzer (Xi'An Remax Electronic Science & Technology Co. Ltd., Xi'An, China) by collecting the maximum ECL intensity during the cyclic potential sweep with a series of optical filters that ranged from 250 to 800 nm. And the scan rate was 0.1 V/s in ECL detection. Cyclic voltammetric (CV) measurements were carried out with a CHI 660D electrochemistry workstation (Shanghai CH Instruments, China). The concentration of Pb^{2+} in cell lysis buffer was calibrated by inductively coupled plasma mass spectrometry (ICP-MS, 7500ce, Agilent, USA). A three-electrode electrochemical cell

was composed of a modified glass carbon electrode (GCE, Φ = 4 mm) as the working electrode, an Ag/AgCl (sat. KCl) as the reference electrode and a platinum wire as the auxiliary electrode. GCE, platinum wire and AgCl electrode were obtained from Tianjin Aidahengsheng technology Co. Ltd., China. The morphologies of different nanomaterial were characterized by scanning electron microscopy (SEM, S-4800, Hitachi, Tokyo, Japan) at an acceleration voltage of 5.0 kV and transmission electron micrograph (TEM) at Tecnai G2 F20 microscope (FEI Co. U.S.A). The UV-vis absorption spectrum were recorded on a UV-2550 spectrophotometer (Shimadzu, Japan). X-ray photoelectron spectroscopy (XPS) characterization was carried out using a VG Scientific ESCALAB 250 spectrometer (Thermoelectricity Instruments, USA) with AlKa X-rays (1486.6 eV) as the light source.

Synthesis of Ag nanoparticles

Ag nanoparticles (AgNPs) were synthesized by a previous literature with a little modification.[1] Firstly, $AgNO_3$ aqueous solution (0.5 mL, 0.01 M) and trisodium citrate dehydrate aqueous solution (1 mL, 0.03 M) were added into ultrapure water (4 °C, 48.5 mL). Then, $NaBH_4$ solution (1.79 mg/mL) was added into the mixture with magnetic stirring. When the color of the solution turned into yellow, AgNPs were well formed.

Prepration of convex Pd@Au HOHs

Pd@Au HOHs were prepared according to a previous literature with some minor modification.[2] Firstly, cubic Pd seeds (PdNCs) with size of 22 nm were synthesized by a simple method. Briefly, 5 mL of K_2PdCl_4 solution (10 mM) was added into 9.42

mL CTAB (12.5 mM) solution. The mixture was kept heating at 95 °C for 5 min with stirring. Then, 80 mL of AA (100 mM) solution was added into the hot mixture and the mixture was kept stirring for 20 min. The obtained PdNCs were purified by centrifuging and washing with CPC aqueous and finally dispersed into 10 mL CPC (5 mM) solution. The Pd@Au HOHs were synthesized by a previous report. Initially, 25 mL of CPC (5 mM) solution was heated at 30 °C for 10 min. After that, 500 μL of the obtained PdNCs, 720 μL of AA (100 mM) and 1500 μL $HAuCl_4$ (10 mM) were added to CPC solution under stirring respectively. The color of CPC solution turned into yellow and finally became orange pink within several minutes, which mean the Pd@Au HOHs were well prepared. The Pd@Au HOHs were collected by centrifugation and washed with ultrapure water two times. Finally, the obtained Pd@Au HOHs were dispersed in 1.5 mL of ultrapure water.

Prepration of Pd@Au HOHs-T_2-S_1 and Pd@Au HOHs-S_2 bioconjugates

Briefly, 800 μL of S_1 (2.5 μM) and 200 μL of T_2 (2.5 μM) were added into 500 μL of the obtained Pd@Au HOHs solution with stirring for 12 h. After this step, amino-modified S_1 and amino-modified T_2 could be immobilized on Pd@Au HOHs to form Pd@Au HOHs-T_2-S_1 through Au-N bond. The Pd@Au HOHs-S_2 bioconjugates were prepared through similar methods. 800 μL of S_2 (2.5 μM) were added into 500 μL of the obtained Pd@Au HOHs solution with stirring for 12 h to form Pd@Au HOHs-S_2 bioconjugates. The obtained bioconjugates were stored at 4 °C before used.

Cell culture and cell lysis buffer preparation

Hela cells were cultured in DMEM medium supplemented with 10% fetal calf

serum containing different concentration of Pb^{2+}. And the Hela cells were maintained at 37 °C in a humidified atmosphere (95% air and 5% CO_2). Cells were collected in the exponential phase and washed twice with ice-cold sterile PBS. After that, 10^6 cells were resuspended in 1 mL of ice-cold sterile water and stored in 2 mL EP tube. Then, the cells were repeatedly frozen and thawed to prepare cell lysis buffer. The cell lysis was used immediately for detection or frozen at -80 °C.

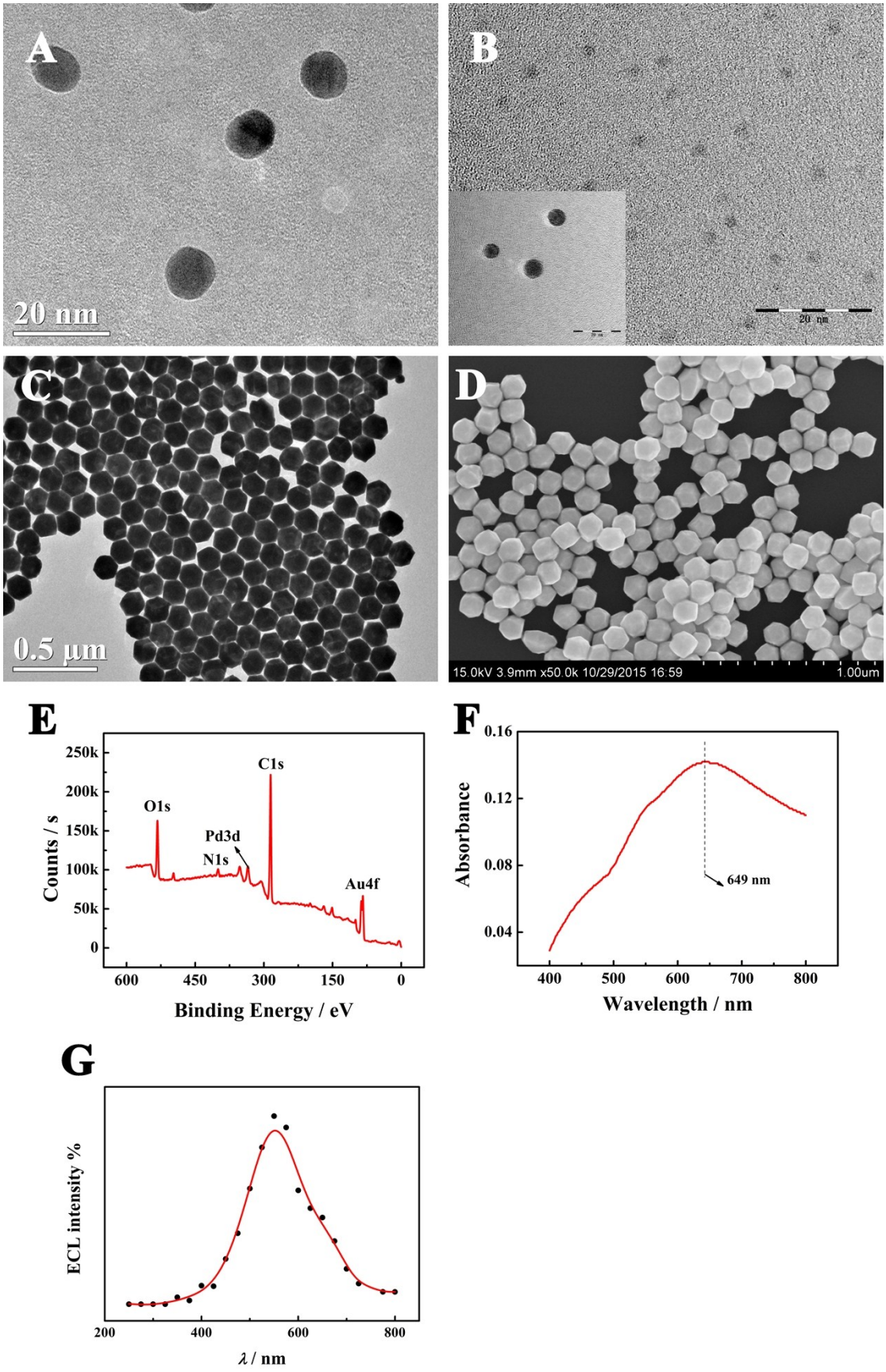
Fabrication of the ECL biosensor

The ECL biosensor was fabricated by the following steps. Initially, the GCE was polished with 0.3 μm and 0.05 μm alumina powder slurry. After being washed with ultrapure water, the GCE was sonicated in ethanol and ultrapure water respectively and dried with nitrogen at room temperature. For electrodepositing of N-CDs, the electrode was immersed in 3 mL of OPD solution (10 mM) for cyclic voltammetric (CV) reaction. The scanning was performed between 0 and 0.6 V at a rate of 50 mV^{-1} for 10 cycles. After that, the N-CDs were deposited on the surface of electrode. Then, 16 μL of AgNPs were dropped onto the GCE and incubated for 12 h. Through Ag-N bond, AgNPs were immobilized on the amino groups of N-CDs. Subsequently, 16 μL of amino modified T_1 (2.5 μM) were dropped onto the GCE and incubated for 12 h to immobilize on the AgNPs through Ag-N bond. Afterward, the modified electrode (T_1/AgNPs/N-CDs/GCE) was incubated in HT (1 mM) at room temperature to block the nonspecific binding sites (HT/ T_1/AgNPs/N-CDs/GCE). Finally, 8 μL of Pd@Au HOHs-T_2-S_1 and 8 μL of Pd@Au HOHs-S_2 were dropped onto the GCE and incubated for 4 h at room temperature. With the chain hybridization between T_1 and

T_2, Pd@Au HOHs-T_2-S_1 was modified on the electrode. Meanwhile, Pd@Au HOHs-DNA dendrimers were formed on the electrode through the chain hybridization between S_1 and S_2 (Pd@Au HOHs-DNA dendrimers/HT/T_1/AgNPs/N-CDs/GCE). Through these steps, the ECL biosensor was well prepared and stored in the refrigerator at 4 °C for further use. After every modified step, ultrapure water was utilized to wash the GCE to remove physically absorbed species.

Measurement Procedure

Firstly, 16 μL of cell lysis buffer were heated 90 °C for 5 min and allowed for slow cooling down to room temperature in 1 h. Then, 16 μL of prepared cell lysis buffer were incubated on the electrode at room temperature for 2 h to form Pb^{2+}-stabilized G-quadruplex (G4). With the forming of Pb^{2+}-stabilized G-quadruplex (G4), the ECL intensity of N-CDs was quenched by Pb^{2+}. After incubation, the modified GCE (Pb^{2+}/Pd@Au HOHs-DNA dendrimers/HT/T_1/AgNPs/N-CDs/GCE) was rinsed with ultrapure water to remove the extra reagents. To measure the ECL response, a conventional three electrode system that used the modified GCE as the working electrode, Ag/AgCl (saturated KCl) as the reference electrode and a platinum wire as auxiliary electrode was investigated in 3 mL of .01 M PBS (pH = 7.4) containing 0.1 M $K_2S_2O_8$. Under CV mode, the working potential scanned from -2.0 to 0 V at a scanning rate of 100 mV/s.



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2 **Fig. S1** (A) TEM image of AgNPs. (B) TEM image of N-CDs. (C) TEM image and (D) SEM

image of Pd@Au HOHs. (E) Full region XPS for Pd@Au HOHs. (F) UV-vis absorption spectra of Pd@Au HOHs. (G) ECL spectrum of N-CDs.

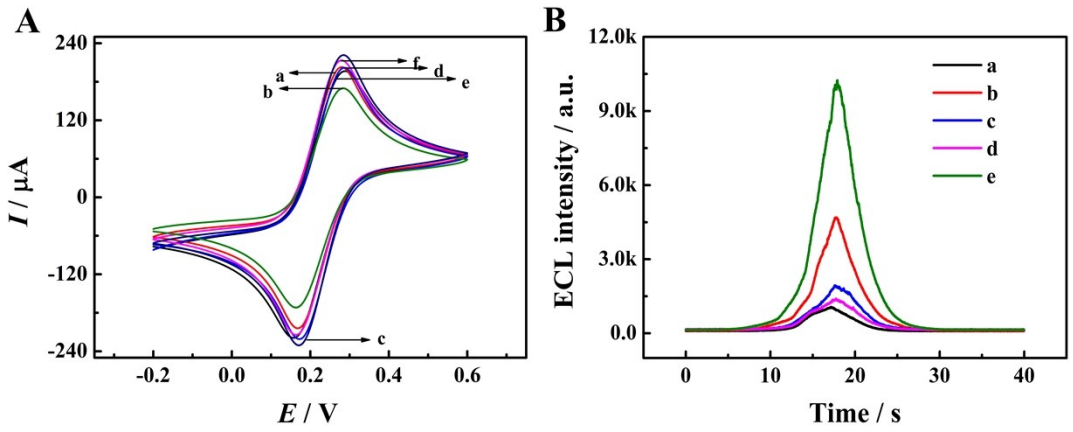

Fig. S2 (A) Cyclic voltammograms at a) bare GCE, b) N-CDs/GCE, c) AgNPs/N-CDs/GCE, d)T_1/AgNPs/N-CDs/GCE, e) HT/T_1/AgNPs/N-CDs/GCE and f) Pd@HOHs-DNA dendrimers/HT/T_1/AgNPs/N-CDs/GCE in 5.0 mm $[Fe(CN)6]^{3-/4-}$ containing 0.1 M KCl by scanning the potential from -0.2 to 0.6 V at a scan rate of 0.1 Vs^{-1}. (B) ECL-time profiles of a) N-CDs/GCE, b) AgNPs/N-CDs/GCE, c) T_1/AgNPs/N-CDs/GCE, d) HT/T_1/AgNPs/N-CDs/GCE and e) Pd@HOHs-DNA dendrimers/HT/T_1/AgNPs/N-CDs/GCE in 0.1 M PBS (pH 7.4) with a scanning potential from 0 to -2 V and at a scan rate of 100 mV s^{-1}.

References

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