

ESI for:

High-density assembly of chemiluminescence functionalized gold nanodots on multiwalled carbon nanotubes and their application as biosensing platform

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Experimental section

Chemicals and Materials

The carboxylated multiwalls carbon nanotubes (MWCNTs) were obtained from Shenzhen Nanotech Port Co., Ltd. (Shenzhen, China). HAuCl_4 stock solution (2% HAuCl_4 , w/w) was prepared by dissolving 1.0 g of $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ (shanghai Reagent, China) in 412 mL of purified water and stored at 4 °C. A 4 mM stock solution of N-(aminobutyl)-N-(ethylisoluminol) (ABEI) was prepared by dissolving ABEI (TCI, Japan) in 0.1 M NaOH solution without further purification. Chitosan ($M_v > 1000$ kDa, degree of deacetylation >90%) was purchased from shanghai Reagent (Shanghai, China) and used as received. Working solution of H_2O_2 were prepared fresh daily from 30% (v/v) H_2O_2 (Xinke Electrochemical Reagent Factory, Bengbu, China). Working solutions of ABEI were prepared by diluting the stock solution. The indium tin oxide (ITO)-coated glass (1.1 mm thick and $<10 \Omega \text{ cm}^{-2}$ resistant) was purchased from CSG Holding Co., Ltd. (Shenzhen, China). Sylgard 184 silicone elastomer and curing agent were obtained from Dow Corning (Midland, MI). All other reagents were of analytical grade and used as received. Ultra-pure water was prepared by a Milli-Q system (Millipore, France) and used throughout.

Synthesis of Chitosan-grafted MWCNTs

All glassware used in the following procedures was cleaned in a path of freshly prepared 3:1 (v/v) HNO_3 -HCl, rinsed thoroughly in redistilled water, and dried prior to use. The Chitosan-grafted MWCNTs (cs-MWCNTs) was synthesized by grafting chitosan onto the carboxylated CNTs in acetic acid aqueous solution at 98 °C for 24 h. In a typical experiment, 2 g of as-received chitosan was added to 200 mL ultra-pure water containing 0.2% v/v of acetic acid. After chitosan was completely dissolved, 200 mg of carboxylated MWCNTs was added to the acetic acid aqueous solution. The solution was theme magnetically stirred and heated to 98 °C to allow grafting reactions for 24 h. After grafting reactions, the solution was carefully centrifuged, followed by washing several times using acetic acid aqueous solution to completely remove free chitosan. Finally, the resulting cs-MWCNTs were dispersed in ultra-pure water solution. This stock solution was stored in the freezer.

Synthesis of ABEI functionalized ABEI-AuNDs-cs-MWCNTs

Firstly, 200 μ L of the cs-MWCNTs stock solution synthesized above was added to 45 mL of ultra-pure water. Secondly, 7 mL of HAuCl₄ (6 mM) solution was added to the mixture and stirring vigorously for 2 h. Then 5 mL of ABEI (4 mM) added to the mixture solution and was stirred for 12 h. Thus, highly density ABEI functionalized AuNDs assembled on the surface of cs-MWCNTs (ABEI-AuNDs-cs-MWCNTs) were obtained. The prepared ABEI-AuNDs-cs-MWCNTs were stored at 4 °C.

Characterization of cs-MWCNTs and ABEI-AuNDs-cs-MWCNTs

As-synthesized cs-MWCNTs and ABEI-AuNDs-cs-MWCNTs were subsequently characterized by transmission electron microscopy (TEM, JEM-ARM200F, Japan), energy dispersive spectroscopy (EDS, JEM-ARM200F, Japan). The histograms were obtained by measuring the diameters of nanoparticles ($n > 100$) in TEM photomicrographs. Through a centrifugation-ultrasonic dispersion process, the inorganic or organic impurities were removed completely. The precipitates were redispersed in ethyl alcohol solution for the TEM detection. The surface composition of cs-MWCNTs and ABEI-AuNDs-cs-MWCNTs were characterized by X-ray photoelectron spectroscopy (XPS) and Raman spectra. The purified cs-MWCNTs and ABEI-AuNDs-cs-MWCNTs were dried under vacuum at room temperature for XPS and Raman detection. Raman spectra were measured using a LABRAM HR (Jobin Yvon, Horiba) with excitation wavelength of 514.5 nm. XPS was performed on an ESCALABMK II electron spectrograph (VG Scientific, UK) with Al K α radiation as the X-ray source. The XPS data were fitted by XPS Peak and further plotted by Origin Pro 7.0.

Fabrication of mercury sensor

ITO-coated glass slides were used as an assembly electrode owing to their stability, easy miniaturization and compatibility with microfabrication. An ITO glass slide with a dimension of 6.0 cm $\times$ 1.5 cm was sonicated alternately with acetone, ultra-pure water and ethanol each for 10 min to get a clean ITO surface. The punched polydimethylsiloxane (PDMS) layer could be immobilized on the surface of ITO, which was prepared by curing the mixture do PDMS monomer and the curing agent

(10:1) in an oven at 75 °C for 1.5 h. The effective electrode area was confined by the punched round hole with a diameter of 5 mm, which was used as a reservoir for subsequent assembly. To exclude the effects of residual reactants and products such as excess ABEI molecules on the ECL measurement, a centrifuging process was necessary to separate these compounds from ABEI-AuNDs-cs-MWCNTs. Typically, 1 mL of ABEI-AuNDs-cs-MWCNTs solution was centrifuged at 8000 rpm for 10 min and then redispersed in the 100 μ L ultra-pure water, thus the inorganic or organic impurities were removed completely. 10 μ L ABEI-AuNDs-cs-MWCNTs solution that described above was dripped onto the surface of ITO glass and dried under an infrared lamp. Thus, ABEI-AuNDs-cs-MWCNTs functionalized ITO surface was obtained.

ECL detection

ECL measurements were carried out with a homemade ECL system consisting of a model CHI760B workstation (Chenhua, Shanghai, China), a model RFL-1 luminometer (Remax, Xi'an, China), an H-type electrochemical cell (self-designed) and a computer. The generation of ECL response comprises electrochemical reactions of the ECL reagent ABEI contained in the ABEI-AuNDs-cs-MWCNTs with H_2O_2 with a conventional three-electrode setup and a light emission process from the excited state of the oxidation products of ABEI to the ground state. The modified part of the ITO electrode was immersed in 1.0 mM H_2O_2 / 20 mM pH 10.18 carbonate buffer solutions (CBS) as working solution. When a double-step potential (20 s pulse period, 0.1 s pulse time, 0.8 V pulse potential and 0 V initial pulse potential) was applied to the working electrode, an ECL signal was generated and recorded. For the sensitivity measurement, different concentration (10, 50, 100, 500, 1000 nM) of Hg^{2+} were used and the ECL signal were recorded. The selectivity was checked by adding of 1.0×10^{-5} M, Fe^{3+} , Fe^{2+} , Cu^{2+} , Cr^{3+} , Co^{2+} , Ni^{2+} , Pb^{2+} , Mn^{2+} , Mg^{2+} and Cd^{2+} instead of 1.0×10^{-6} M Hg^{2+} in the sensing protocol

CL detection of ABEI-AuNDs-cs-MWCNTs

The static injection CL detection was conducted on a BPCL Luminescence Analyze (Beijing, China) with a fixed voltage of -800 V. For a typical CL measurement, 100

μL of the centrifuged ABEI-AuNDs-cs-MWCNTs was added to a cylindrical cell, then 50 μL of 0.1 M H_2O_2 containing 0.1 M NaOH solution was injected into the cell to initiate the CL reaction. The CL intensity during the reaction along with time was recorded by the Luminescence Analyzer.

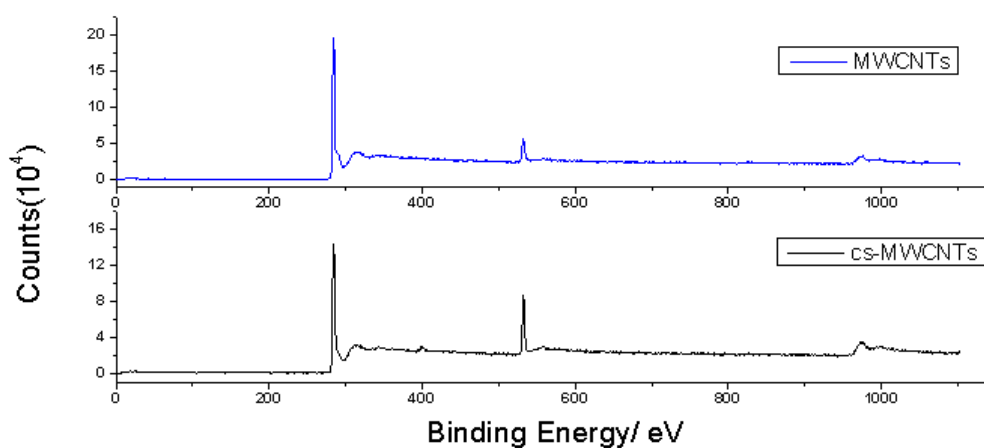


Fig. S1 XPS survey spectra of MWCNTs and cs-MWCNTs.

XPS was performed to characterize the surface state of MWCNTs and cs-MWCNTs and the results are shown in Fig. S1. Compared with MWCNTs, the survey of cs-MWCNTs showed a peak around 400 eV and it was corresponding to the N 1s. Thus the XPS results confirmed that there was a new component containing nitrogen in cs-MWCNTs, indicating the existence of chitosan molecules on the surface of cs-MWCNTs and the successful functionalization of MWCNTs by chitosan.

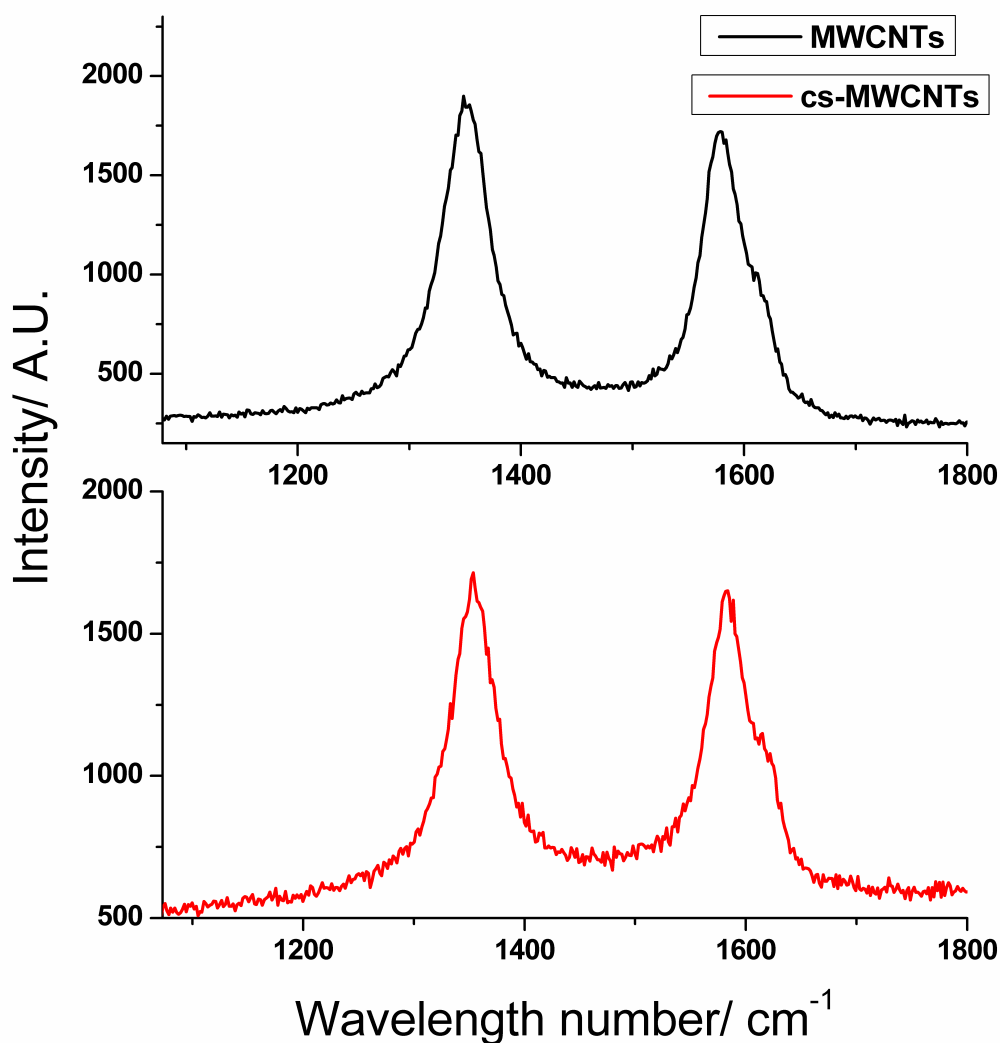


Fig. S2 Raman spectra of MWCNTs and cs-MWCNTs.

Raman spectroscopy is an efficient tool to characterize MWCNTs-based materials. It was used to monitor the structural changes occurring during the synthesis of cs-MWCNTs. The Raman spectrum of MWCNTs showed two peaks, centered at 1578 and 1349 cm⁻¹, which were attributed to G and D bands, respectively. Similarly, the cs-MWCNTs also contained both G and D bands with a lower D/G intensity ratio as compared with that of MWCNTs. This result revealed that the reduction of the degree of disorder at the surface of cs-MWCNTs and was in good agreement with the previous report, which indicating that chitosan was grafted on the surface of MWCNTs in this work.

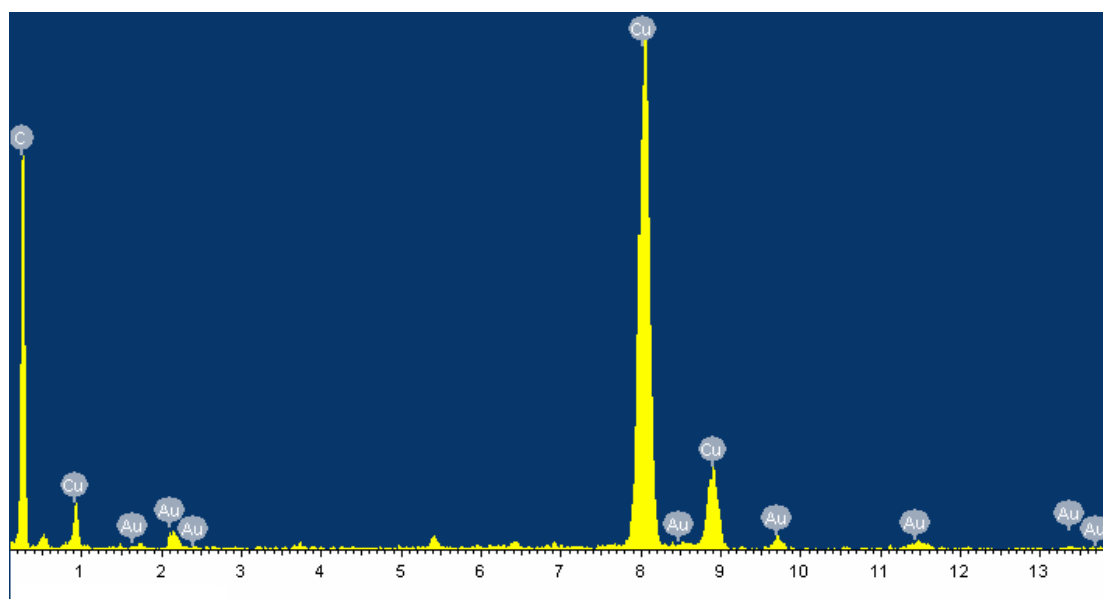


Fig. S3 EDS spectrum of ABEI-AuNDs-cs-MWCNTs

The EDS result of ABEI-AuNDs-cs-MWCNTs is shown in Fig. S3 and the peaks were labelled with the lines of the corresponding elements. EDS spectrum of ABEI-AuNDs-cs-MWCNTs was carried out on the copper grid, and all reagents used in this experiments did not contains copper element, thus the peaks corresponding to the copper elements was resulted from the copper grid. This result revealed that the main element existed on the surface of ABEI-AuNDs-cs-MWCNTs was Au.

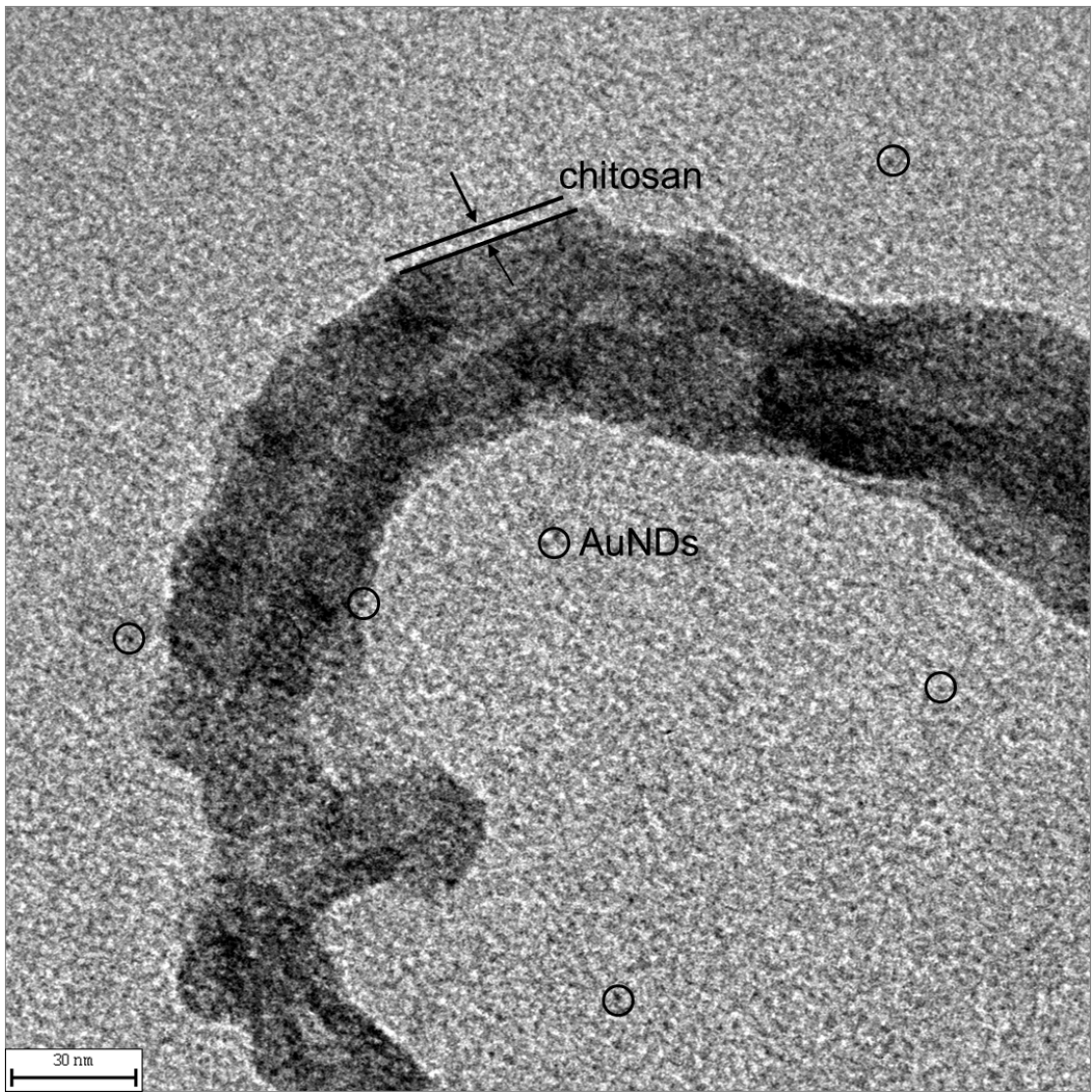


Fig. S4 TEM image of cs-MWCNTs mixed with pre-synthesized ABEI-AuNDs

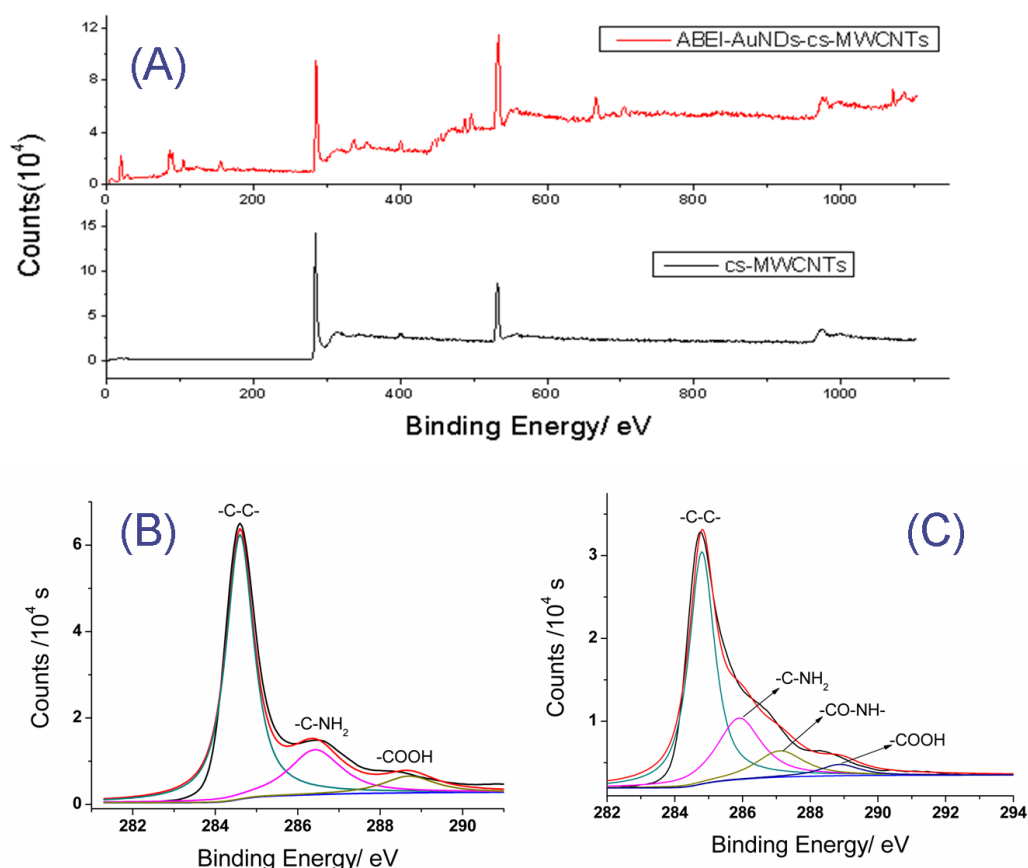
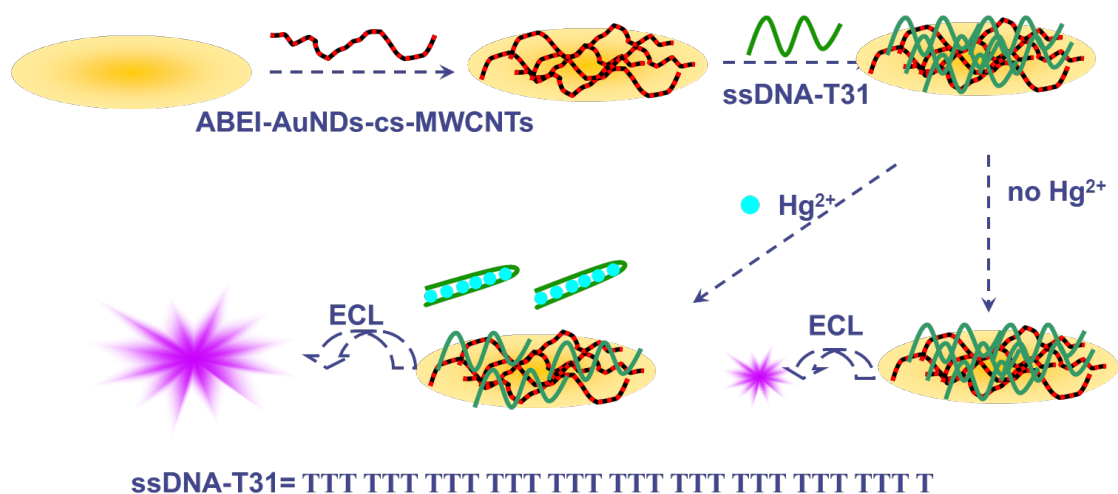


Fig. S5 (A) XPS survey spectra of cs-MWCNTs and ABEI-AuNDs-cs-MWCNTs. (B) XPS spectrum of C 1s from cs-MWCNTs. (C) XPS spectrum of C 1s from ABEI-AuNDs-cs-MWCNTs.



Scheme S1 Schematic illustration for fabrication of label-free ECL aptasensor towards Hg^{2+}

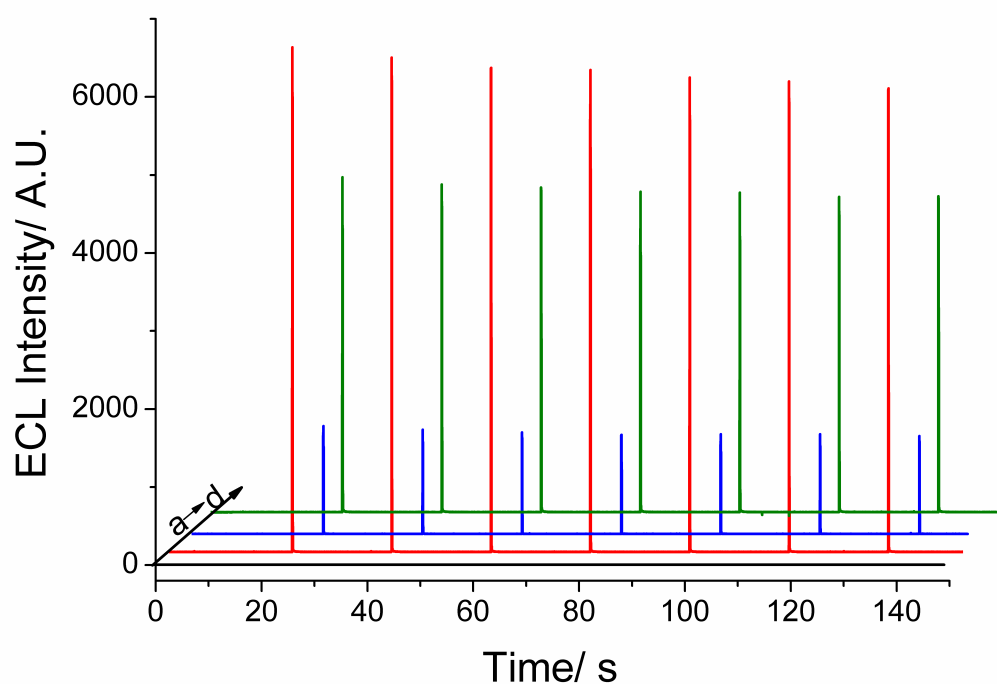


Fig. S6 ECL signals under pulse potential obtained (a) on a ITO substrate, (b) on a TIO/ABEI-AuNDs-cs-MWCNTs substrate, (c) on a ITO/ABEI-AuNDs-cs-MWCNTs/aptamer substrate, (d) ITO/ABEI-AuNDs-cs-MWCNTs/aptamer substrate after adding the target Hg^{2+} (1.0×10^{-6} M). Initial potential, 0 V; pulse potential, 0.8 V; pulse period, 20 s; pulse time, 0.1 s; H_2O_2 1 mM; CBS, 0.02 M (pH 10.18).

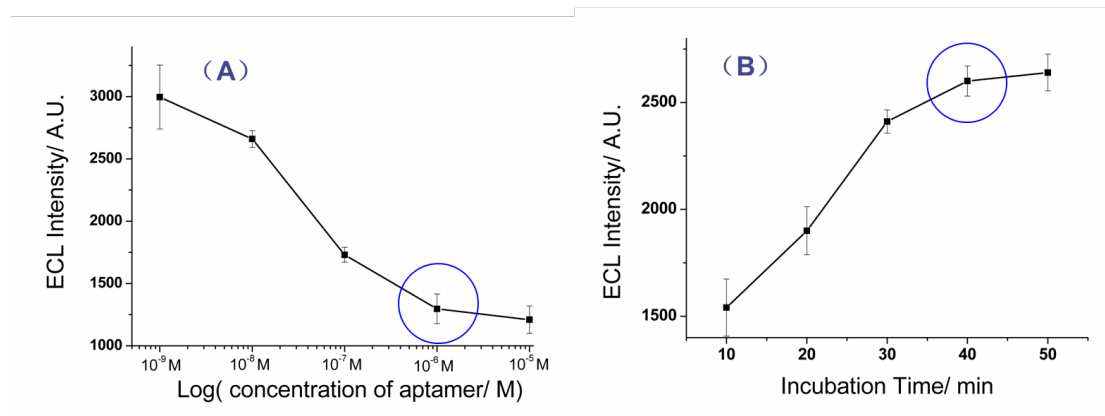


Fig. S7 Effects of concentration of aptamer and incubation time for aptamer-target interaction on ECL intensity.

In order to obtain the best sensitivity and selectivity, two important parameters, including the concentration of aptamer and the incubation time, were optimized as shown in Fig. S7. The influence of concentration of aptamer in the range of 1.0×10^{-5} – 1.0×10^{-9} M on the ECL intensity was examined. As shown in Fig. S7 (A), the ECL intensity decreased with the increasing of the concentration of aptamer, indicating the aptamer successfully adsorbed on the surface of ABEI-AuNDs-cs-MWCNTs through electrostatic interaction. The ECL intensity decreased obviously from 1.0×10^{-9} M to 1.0×10^{-6} M and had little change from 1.0×10^{-6} M to 1.0×10^{-5} M. Considering cost, the concentration of aptamer was 1.0×10^{-6} M for the following experiments.

Incubation time for the aptamer-target interaction greatly influenced the sensitivity of the developed aptasensor. The effect of incubation time on ECL intensity was investigated. As shown in Fig. S7 (B), ECL intensity increased with the increasing incubation time and trended to level off after 40 min, and longer incubation time could not improve the response. Thus 40 min of incubation time for the aptamer-target interaction was chosen for the following experiments.

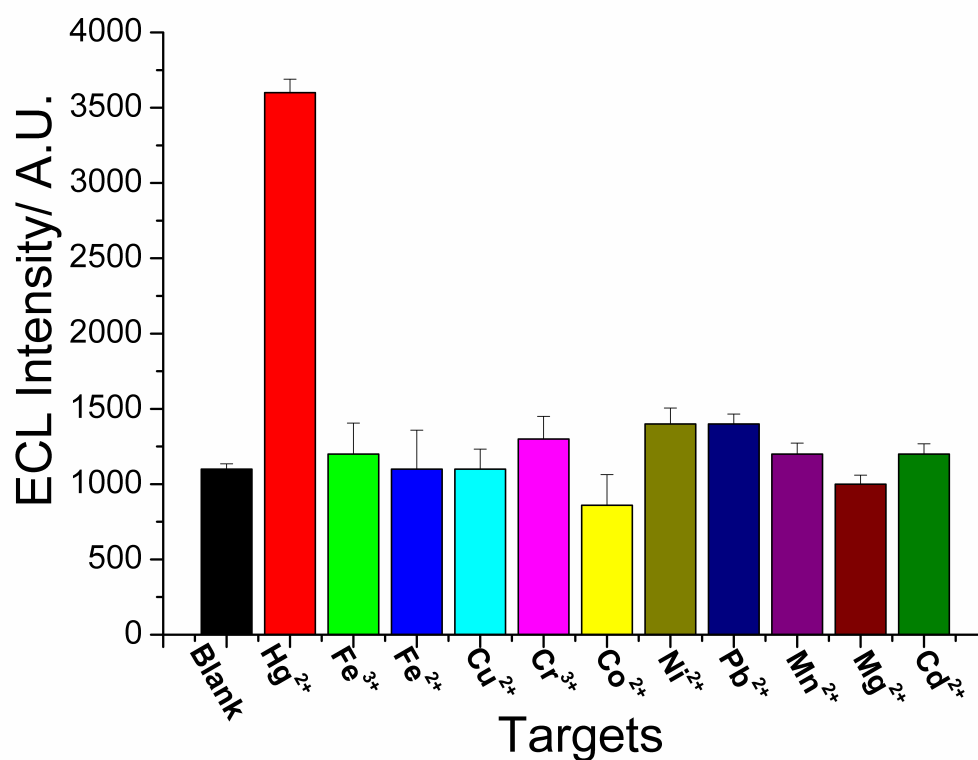


Fig. S8 A comparison of ECL responses of Hg²⁺ with those of different interfering species. Initial potential, 0 V; pulse potential, 0.8 V; pulse period, 20s; pulse time, 0.1 s; H₂O₂ 1 mM; CBS, 0.02 M (pH 10.18).

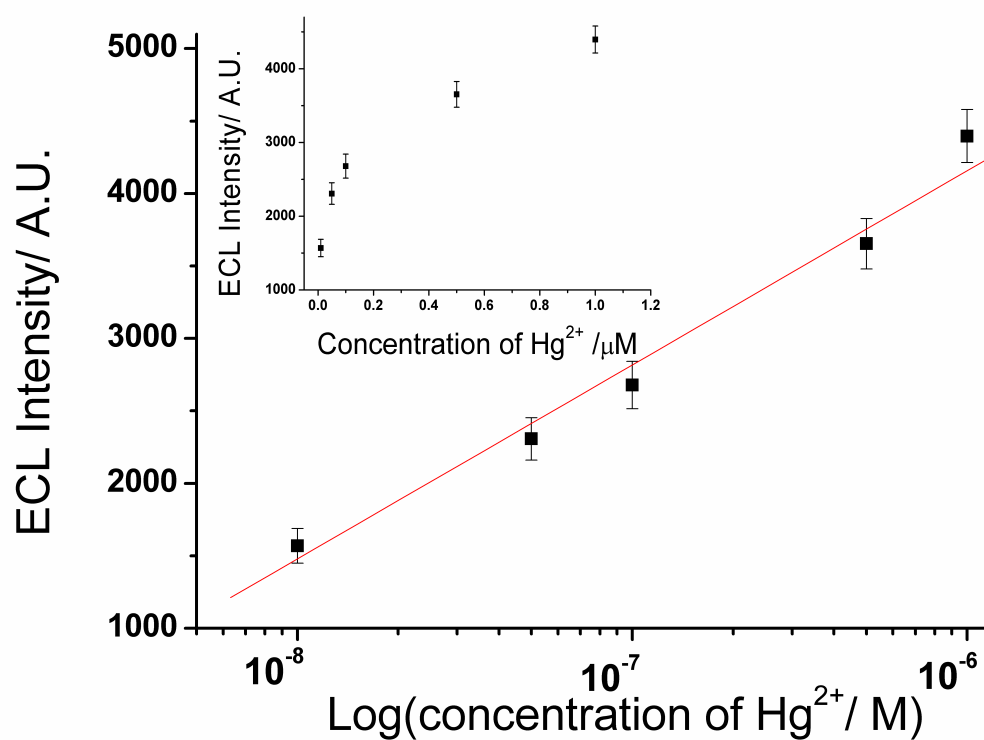


Fig. S9 Calibration curve of ECL intensity versus concentration of Hg^{2+} in water samples (obtained for the Yexi Lake, Hefei, China). Conditions: initial potential, 0 V; pulse potential, 0.8 V; pulse period, 20s; pulse time, 0.1 s; H_2O_2 1 mM; CBS, 0.02 M (pH 10.18).

Table S1 Determination of Hg^{2+} in water samples with the aptasensor.

Water samples	Hg^{2+} found	Hg^{2+} added concentration (1.0×10^{-7} M)	Hg^{2+} detected concentration (1.0×10^{-7} M)	Recovery (%)	RSD (%) n = 5
1	--	0.1	0.095	95	5.9
2	--	2.0	2.3	115	4.2
3	--	6.0	6.7	112	3.3

--: No Hg^{2+} ions were found in original water samples.