

Electronic Supplementary Information (ESI) for Chemical Communications

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Chemiluminescence excited photoelectrochemistry using graphene-quantum dots nanocomposite for biosensing

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Experimental

Materials and reagents. Luminol ($\geq 97\%$), chitosan (CS) (viscosity > 200 cP), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich Inc. (USA). Glutaraldehyde (GLD) (28%) and *p*-iodophenol (PIP) (98%) were purchased from Alfa Aesar China Ltd. (China). Chloroauric acid ($\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$) was obtained from Shanghai Reagent Company (China). Carcinoembryonic antigen (CEA), monoclonal Ab₁ and horseradish peroxidase (HRP) labeled anti-CEA Ab₂ were purchased from Beijing Keybiotech Co., Ltd (China). Fluorine-doped tin oxide (FTO) electrode was purchased from LOF glass company (USA). All other chemicals were of analytical grade. Tris-HCl buffered saline (0.1 mol L^{-1} , pH 8.5) was employed as supporting electrolyte. The washing solution was 0.01 M pH 7.4 phosphate buffered saline (PBS). 0.01 M pH 7.4 PBS containing 1% (w/v) BSA was used as blocking solution. Ultrapure water obtained from a Millipore water purification system ($\geq 18 \text{ M}\Omega$, Milli-Q, Millipore) was used in all assays.

Blood samples from Jiangsu Hospital of Cancer were centrifuged at 4000 rpm for 5 min to obtain the supernates as clinical serum samples.

Apparatus. Transmission electron micrographs (TEM) were obtained using a JEM-2100 TEM (JEOL, Japan). Resonance Raman spectra were recorded on a Renishaw-inVia Raman microscope (Renishaw, United Kingdom). X-ray photoelectron spectroscopic (XPS) measurements were performed using an ESCALAB 250 spectrometer (Thermo-VG Scientific, USA) with an ultra-high vacuum generator. Fourier-transform infrared spectra (FTIR) were obtained on a Nicolet iS10 instrument (Nicolet, USA). Photoelectrochemical measurements were performed with a home-built photoelectrochemical system. All experiments were carried out at room temperature using a conventional three-electrode system with a modified FTO electrode as working, a platinum wire as auxiliary, and a saturated calomel electrode as reference electrodes.

Preparation of RGO-CdS nanocomposite, and luminol and HRP-Ab₂ functionalized AuNPs.

Graphene oxide (GO) was synthesized from graphite by a modified Hummers method.^{S1} The RGO-CdS nanocomposite was prepared with the GO by a one-pot reaction.^{S2} In brief, about 250 mg of GO was dispersed in 50 mL of 0.035 M Cd(NO₃)₂ by sonication for 30 min. H₂S gas was then bubbled through this dispersion for 30 min. The resulting solid was filtrated, washed several times with water followed by acetone, dried in air at 65 °C, and heated at 100 °C in N₂ atmosphere for 6 h to obtain RGO-CdS nanocomposite. Here H₂S was produced by the reaction of Na₂S with dilute H₂SO₄ and acted as a sulphide source and a reducing agent of GO to form RGO-CdS nanocomposite.

The luminol functionalized AuNPs were prepared by reducing AuCl₄⁻ ions with luminol solution. 100 mL HAuCl₄ solution (0.01%, w/w) was heated to boiling point. While stirring vigorously, 1.55 mL luminol solution (0.01 mol L⁻¹) was added rapidly. The solution was

maintained at boiling point for 30 min. After cooled, the unreacted reagents were removed via dialysis for 3 days with 3500 molecular weight dialysis membrane in ultrapure water.^{S3} The colloidal solution was stored at 4 °C. 500 µL HRP labeled Ab₂ (100 µg mL⁻¹) was added in 1 mL luminol functionalized AuNPs (pH 8.0), stirring at room temperature for 30 min. After centrifugation at 10000 rpm for 20 min at 4 °C, the sediment was resuspended in 1 mL PBS (0.01 M, pH 7.4) to obtain luminol and HRP labeled Ab₂ functionalized AuNPs. To obtain the optimum CL emission and response, here the HRP-labeled Ab₂ was excessive, leading to full coverage of HRP-labeled Ab₂ on the AuNPs at a stirring time of 30 min.

Preparation of photoelectrochemical biosensor. The FTO electrode was cleaned with NaOH (1 mol L⁻¹) and H₂O₂ (30%), then washed with acetone and water, and dried at room temperature. 20 µL of RGO-CdS nanocomposite ethanol solution (5 mg mL⁻¹) was coated on the FTO electrode and dried in shade at room temperature with the thickness of around 200 µm. 20 µL CS solution (0.5 wt%) in 1% acetic acid was then dropped on RGO-CdS coated FTO electrode and dried at 50 °C. After the electrode was washed with 0.1 mol L⁻¹ NaOH solution and ultrapure water, respectively, 20 µL GLD solution (5.0%) was dropped on the electrode for 30 min at room temperature. The activated CS membrane was then bound to anti-CEA antibody by dropping 20 µL antibody solution (100 µg mL⁻¹, pH 7.4) and incubation at 4 °C for 12 h. Finally the electrode was rinsed with a washing buffer and incubated with a BSA blocking buffer for 1 h to block possible remaining active sites against nonspecific adsorption. After rinsing with 0.01 M PBS, the biosensor was obtained.

Measurement procedure. To carry out the CL excited photoelectrochemical measurement, the biosensor was first incubated with 20 µL CEA solution for 35 min at 37 °C, followed by washing with 0.01 M PBS and further incubation with 20 µL luminol and HRP labeled Ab₂ functionalized

AuNPs for 35 min at 37 °C. The biosensor was then inserted in 0.1 M pH 8.5 tris-HCl buffer containing 5 mM H₂O₂ and 0.6 mM PIP to record the photoelectrochemical response at an applied potential of +0.2 V for immunoassay without deaeration.

Characterization

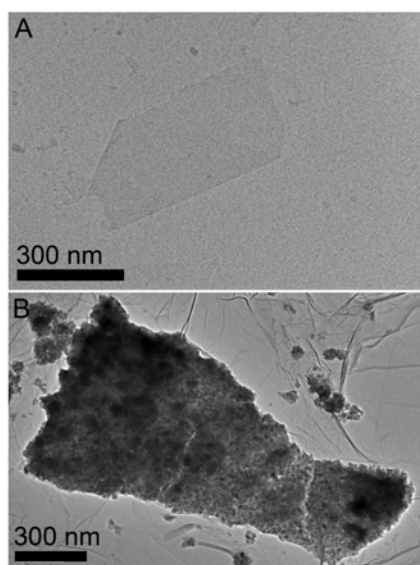


Fig. S1. TEM images of (A) GO and (B) RGO-CdS nanocomposite.

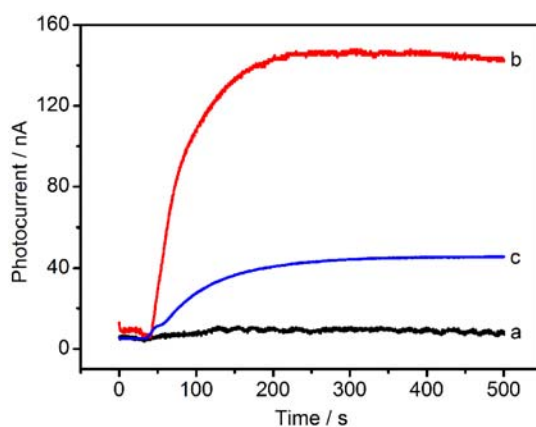


Fig. S2. Photocurrent responses of (a) and (b) RGO-CdS nanocomposite and (c) CdS QDs modified electrode without (a) and with (b) and (c) 0.5 ng mL⁻¹ CEA in 0.1 M pH 8.5 tris-HCl buffer containing 5 mM H₂O₂ and 0.6 mM PIP at +0.2 V.

CL spectrum

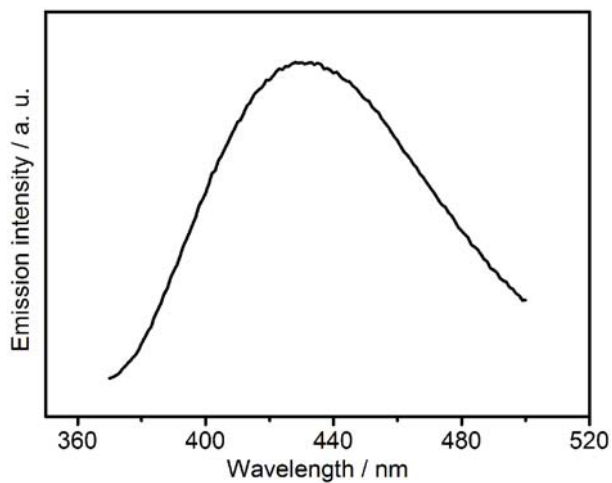


Fig. S3. CL spectrum of luminol-H₂O₂-HRP-PIP system.

Photoelectrochemical response

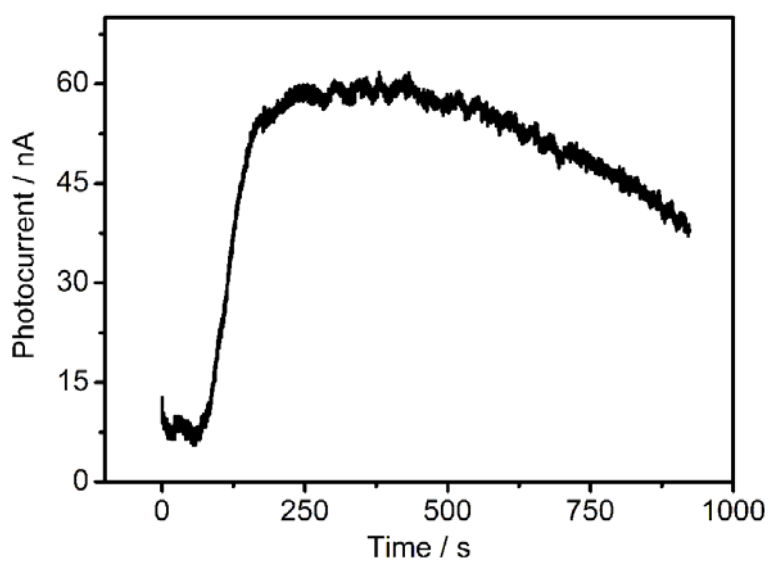


Fig. S4. Photoelectrochemical response of the immunosensor to 0.1 ng mL⁻¹ CEA in 0.1 M pH 8.5 tris-HCl buffer containing 5 mM H₂O₂ and 0.6 mM PIP at +0.2 V.

Optimization of detection conditions

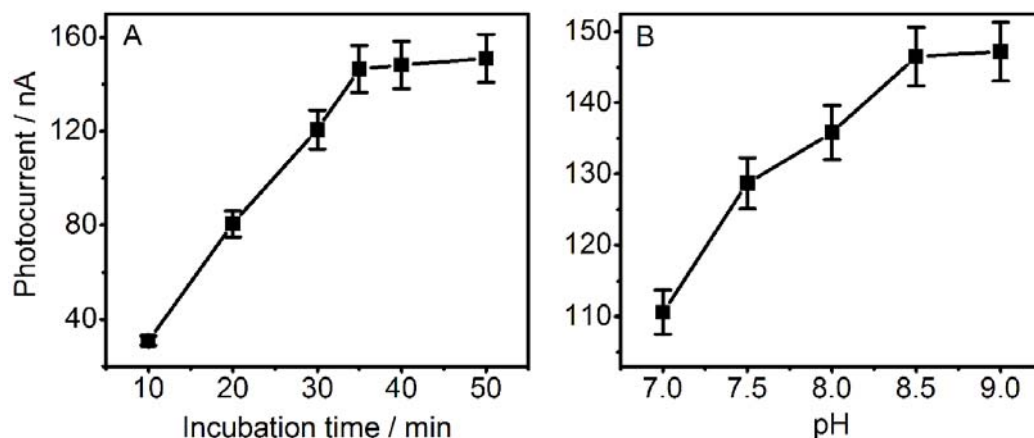


Fig. S5. Effects of (A) incubation time and (B) pH of detection solution on photocurrent response.

At room temperature, the photocurrent response to CEA increased with the increasing incubation time used in sandwich-type immunoassay and then tended to constant value after 35 min (Fig. S5A), which indicated the saturated formation of the sandwich immunocomplex. Therefore, an incubation time of 35 min was selected for the sandwich-type immunoassay.

The photocurrent response increased with the increasing pH value of detection solution and then reached a plateau at pH 8.5 (Fig. S5B). Taking into account the bioactivity of immunoreagents, pH 8.5 was selected.

The applied potential was an important parameter for producing the photocurrent. As seen in Fig. S6, with the increasing potential from 0 to +0.2 V, a sharp increase of photocurrent was observed, which then trended a relatively stable value. The photocurrent at +0.2 V was 87.3% of that at +0.4 V, showing enough sensitivity for photoelectrochemical detection of CEA. The low applied potential was beneficial to the elimination of interference from other reductive species

coexisted in the samples. Thus +0.2 V was chosen for photoelectrochemical determination of CEA.

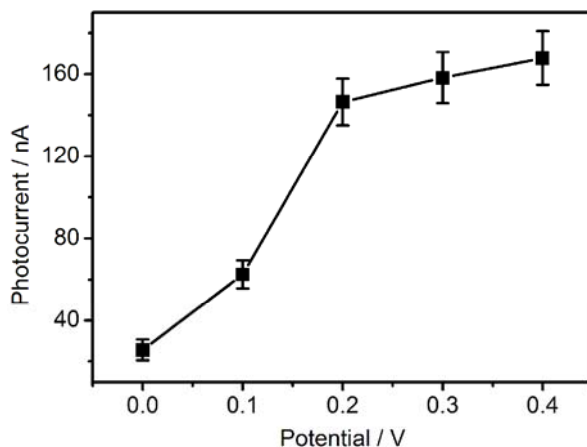


Fig. S6. Effect of the applied potential on photocurrent responses.

Immunoassay results of serum samples

Table S1. Comparison of CEA determinations in human serum samples using the proposed and reference methods.

Sample No	1	2	3	4	5
Proposed method (ng mL ⁻¹)	1.38	1.96	3.31	2.74	63.56
Reference method (ng mL ⁻¹)	1.54	2.11	2.95	3.20	59.67
Relative error (%)	-10.4	-7.11	12.2	-14.4	6.52

This method could be used for clinical sample detection. When the level of serum tumor marker was over the calibration range, serum sample was appropriately diluted with 0.01 M pH 7.4 PBS prior to assay. The assay results of clinical serum samples, compared with the reference

values obtained by commercial electrochemiluminescent test, showed an acceptable agreement, with relative errors less than 14.4% (Table S1), indicating acceptable accuracy.

References

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- S3 Y. Chai, D. Y. Tian, W. Wang and H. Cui, *Chem. Commun.*, 2010, 7560.