Supporting Information

Chemical and Electrochemical Reactions of Coreactants Syner-gistically Mediated Enhancement of Cathodic and Anodic Elec-trochemiluminescence of Graphene Quantum Dots

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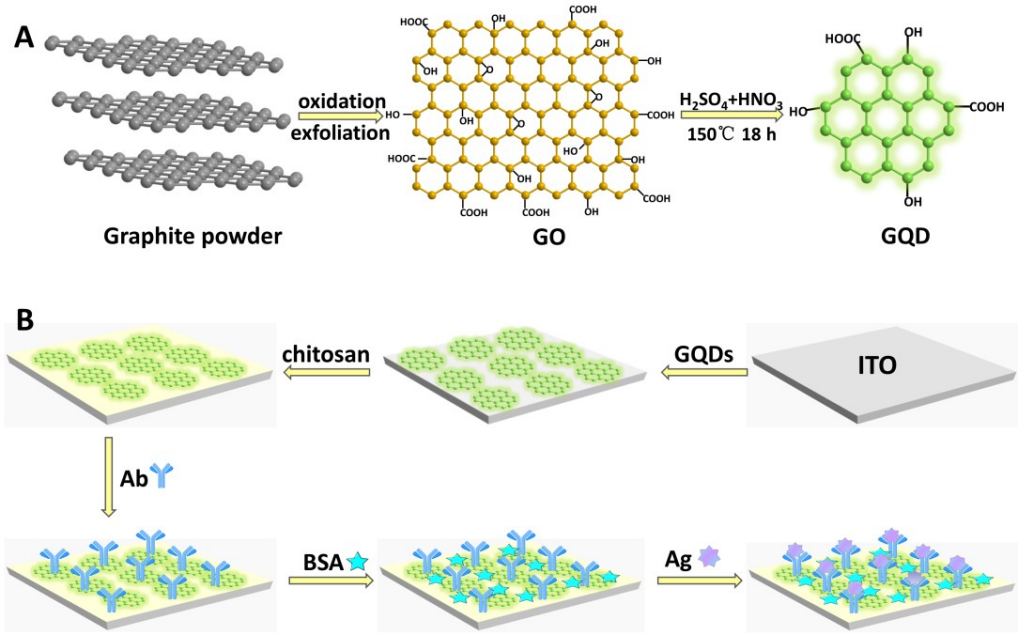
Experimental Procedures

Reagents. Indium tin oxide (ITO)-coated glass slide (thickness: 185±2 nm, resistance: 10 Ω/sqr) was purchased from Southern China Xiang Science and Technology Co. Ltd. (Shenzhen, China). Graphite powder (99.9995%, 100 mesh) was purchased from Alfa Aesar Company (UK). Bovine serum albumin (BSA) was bought from Biosharp Co. Ltd. (China). Glutaricdialdehyde, Ceric ammonium nitrate, potassium persulfate and sodium sulfite were purchased from Sinopharm Chemical Reagent Co. (China). Human IgG (Ag) and rabbit anti-human IgG (Ab) were purchased from Boster Co. Ltd. (Wuhan, China). All other reagents were of analytical-reagent grade. A 0.1M phosphate buffer solution (PBS pH 7.4 containing 0.1 M NaCl) was prepared by mixing 0.1 M Na_2HPO_4 and 0.1 M NaH_2PO_4. All the solutions were prepared with ultrapure fresh water (resistivity of 18.2 MΩ·cm).

Apparatus. Ultraviolet-visible spectra (UV-vis) were obtained on a Nanodrop-2000C spectrophotometer (Thermo Fisher Scientific Company, USA). Photoluminescence spectra (PL) were recorded on a RF-5301PC spectrophotometer (Shimadzu, Japan). Fourier transform infrared spectra (FT-IR) were collected on a Nicolet 6700 FT-IR spectrometer (Thermo Scientific, USA). Electron paramagnetic resonance (EPR) characterization was performed on an EMX-10/12 spectrometer (Bruker Company, Germany). Morphologies of samples were characterized by a transmission electron microscopy (TEM) (JEM-2100, JEOL Company, Japan). Electrochemical impedance spectra (EIS) were measured on a CHI 660E electrochemical workstation (Shanghai Chenhua Instrument, China). Electrochemical and ECL experiments were carried out on an MPI-E ECL analyzer system (Xi'an Remex Electronic Science and Technology Co. Ltd., China) equipped with a PMT of 800 V. The ECL spectra were obtained by collecting the ECL peak intensity during potential sweep with 11 pieces of optical filters at 400, 440, 460, 480, 500, 520, 540, 560, 580, 600 and 620 nm, respectively. All electrochemical experiments were performed with a conventional three-electrode system, including an ITO working electrode, a platinum counter electrode, and an Ag/AgCl (saturated KCl solution) reference electrode. The working area of ITO electrodes was a rectangular shape with dimensions of 15 x 15 x 1.1 mm, and the square resistance is no more than 6 Ω. GQDs were drop cast on ITO surface with a loading amount of 1.5 x 10^{-3} mg.



Scheme S1. (A) Preparation strategy of GQDs, and (B) the fabrication procedure of the ECL immunosensor.

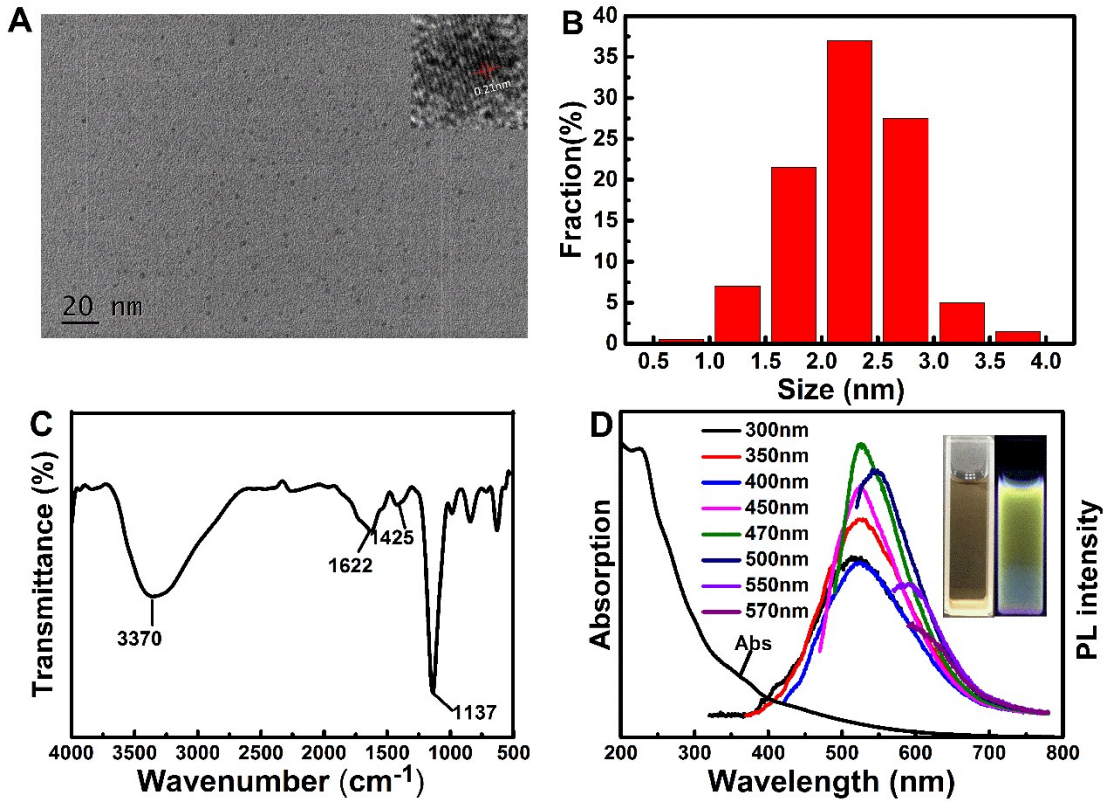


Figure S1. (A) HRTEM image of GQDs. (B) Plot of the size distribution of GQDs. (C) FTIR spectrum of GQDs. (D) UV-vis absorption (Abs, Black) and PL spectra of the GQDs at different excitation wavelengths. Inset of (D) shows the photographs of the GQDs aqueous solution taken under daylight and 365 nm UV light, respectively.

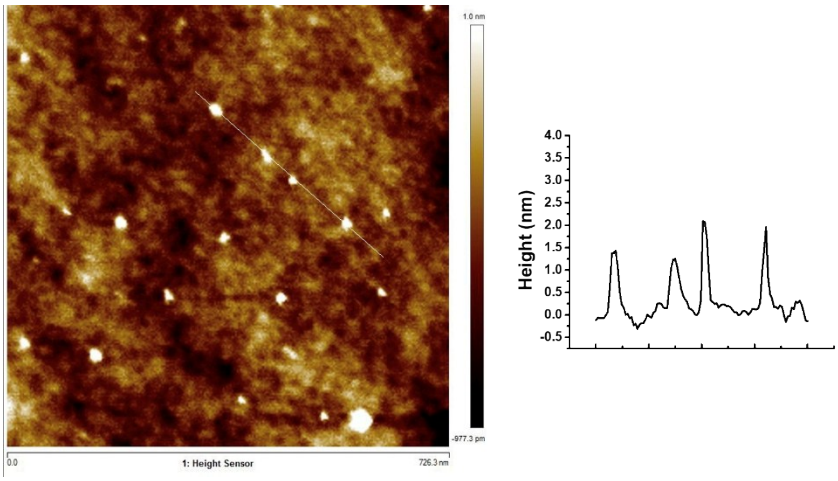


Figure S2. Atomic Force Microscopy (AFM) of the as-synthesized GQDs film deposited on ITO electrode (left) and the corresponding height profile of GQDs film at the position lined in the left panel (right).

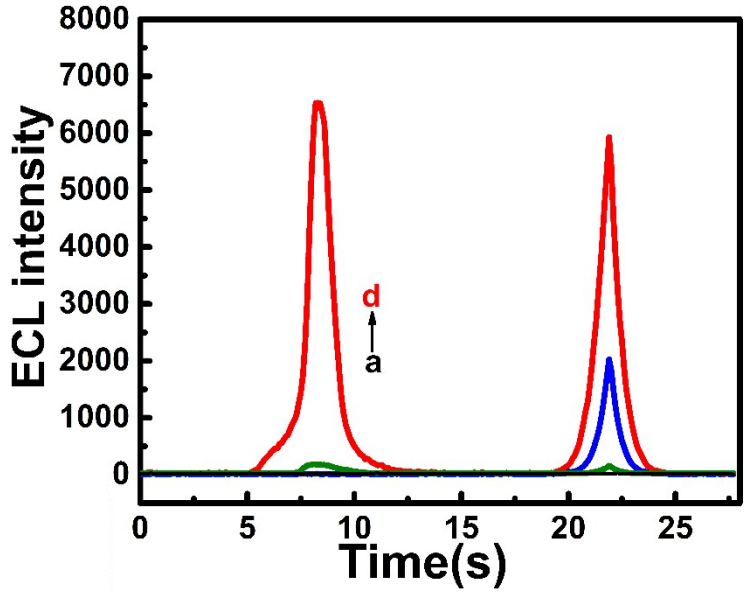


Figure S3. ECL-time profiles of GQDs-100 mM Na_2SO_3 (black line), GQDs-10 mM $K_2S_2O_8$ (blue line), 100 mM Na_2SO_3-10 mM $K_2S_2O_8$ (green line), GQDs-10 mM $K_2S_2O_8$-100 mM Na_2SO_3 (red line) in a 0.1 M air-saturated PBS (pH 7.4) at a scan rate of 0.2 V/s.

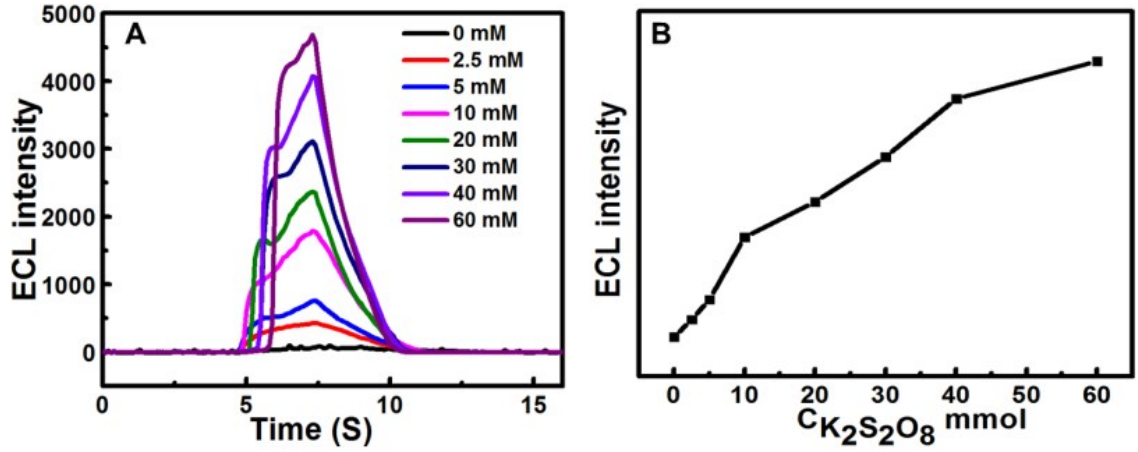


Figure S4. (A) ECL-time profiles of the GQDs-$K_2S_2O_8$-Na_2SO_3 system. The $K_2S_2O_8$ concentration was 0, 2.5, 5, 10, 20, 30, 40 and 60 mM from down to up, and the Na_2SO_3 concentration was fixed at 100 mM with the same GQDs concentration. (B) Influence of the $K_2S_2O_8$ concentration on the anodic ECL signal with the Na_2SO_3 concentration fixed at 100 mM.

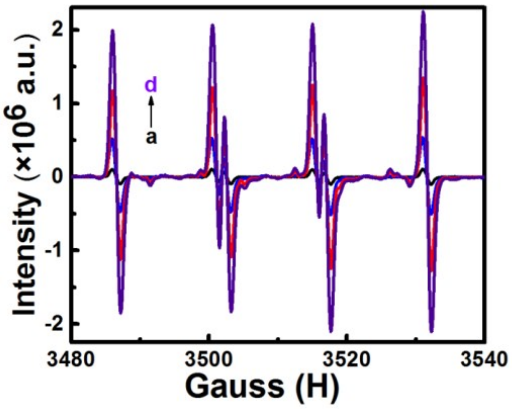


Figure S5. EPR spectra of 0.1 M PBS buffer (pH 7.4) containing (a) 5.0 mM $K_2S_2O_8$, (b) 20.0 mM $K_2S_2O_8$, (c) 50.0 mM $K_2S_2O_8$, and (d) 100.0 mM $K_2S_2O_8$ in the presence of 0.1 M Na_2SO_3 and 0.1 M DMPO.

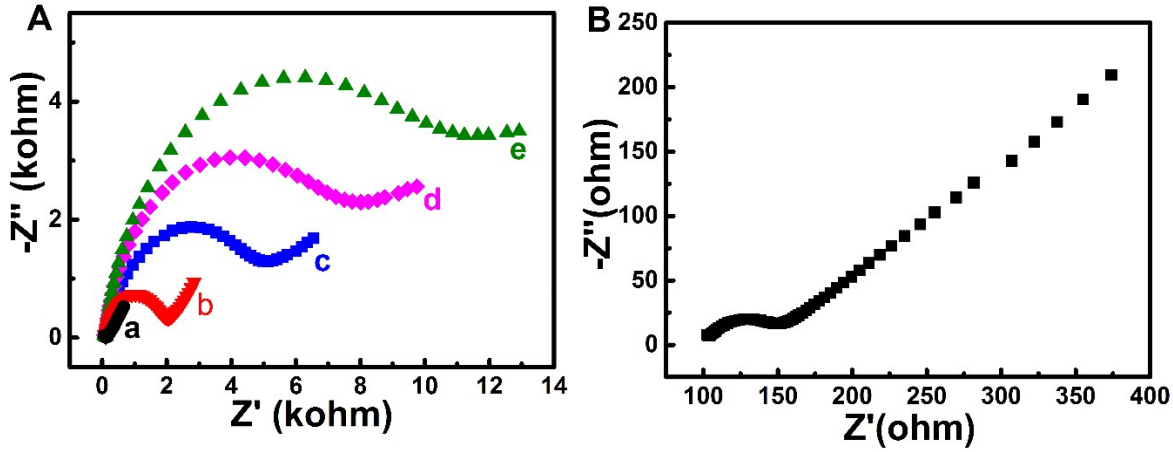


Figure S6. (A) EIS profiles of (a) bare ITO electrode, (b) GQDs/Chitosan, (c) GQDs/Chitosan/Ab, (d) GQDs/Chitosan/Ab/BSA and (e) GQDs/Chitosan/Ab/BSA/Ag in 1 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl. The frequency range was 0.01-1000000 Hz with a signal amplitude of 5 mV. (B) amplified plot of (a).

ECL immunosensor based on GQDs. As shown in Figure S5, an ECL immunosensor based on GQDs was proposed. The stepwise assembly process of the ECL immunosensor was monitored by electrochemical impedance spectroscopy (EIS) in a 1 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.1 M KCl. Figure S5 shows the EIS of the electrode at different stages. It can be seen that bare ITO electrode reveals a small semicircle domain (curve a), which demonstrates the characteristics of a diffusion-controlled electrochemical process. After the electrode is modified with GQDs and chitosan film (CS), the EIS exhibits an increased resistance for the redox probe (curve b) because GQDs-chitosan film inhibits the access of the redox probe to the electrode. After antibody is covalently conjugated to the CS film, the electron-transfer resistance significantly increases due to the insulating antibody layer which inhibits electron exchange between the redox probe and the electrode. When BSA is subsequently assembled onto the electrode, a further increase of the electron transfer resistance occurs (curve d). Finally, the successful capture of antigen leads to an increase of resistance due to steric hindrance of the antigen-antibody complex (curve e). The EIS results prove that the immunosensor has been successfully fabricated.