Supplementary Information

Detection of Ovarian Cancer Biomarker CA-125 by Chemiluminescence Resonance Energy Transfer to Graphene Quantum Dots

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Experimental Methods

Chemicals and Materials: Ovarian cancer antigen CA-125, CA-125 capture antibody (cAb) and CA-125 detection antibody were purchased from Genway Biotech (San Diego, CA). 3-aminopropyl-trimethoxysilane (APTMS), 1-ethyl-3-dimethylaminopropyl carbodiimide (EDC), and N-hydroxysuccinimide (NHS), tris(hydroxymethyl), bovine serum albumin (BSA) fraction powder, PBS (10mM, with 0.137 M NaCl and pH=7.0), methanol/HCl solution, acetic acid (HAc) solution, horseradish peroxidase (HRP) enzyme, luminol and hydrogen peroxide (H_2O_2) were purchased from Sigma-Aldrich (MO, USA). Human Plasma was purchased from Innovative Research (Novi, MI). The quenching buffer was from Ocean NanoTech, LLC (Springdale, AR, USA). Aqueous solutions were prepared with high-purity deionized (DI) water from a Milli-Q Millipore filtration system (18.2 MΩ·cm, Millipore Corp., USA). Glass slides (25mm×75mm) were from Thermo Fisher Scientific Inc. (MA, USA).

Synthesis of GQDs: GQDs were synthesized following the photo-Fenton method described previously [S1]. Briefly, 25 mL of 0.5 mg/mL GO water suspension was mixed with 400 μL of H_2O_2 and 100 μL of 1.0 mM $FeCl_3$ in a quartz tube under vigorous stirring. Photo-Fenton reaction of GO was then carried out by exposing the quartz tube to a mercury lamp (365 nm, 1000 W) for 40 min. The as-generated GQDs were dialyzed in ultrapure water for 2 days prior to use.

Immobilization of GQDs on the glass chip: A glass chip was sonicated in 95% ethanol and deionized (DI) water for 5 min each, respectively, and then immersed in 1:1 methanol/HCl for 30 min, and air-blow dried to create a hydrophilic surface. The chip was then immersed in 5% 3-aminopropyl-trimethoxysilane (APTMS) in methanol at room temperature overnight to functionalize the surface with the amine group. After rinsing with ethanol and DI water, 1 mM acetic acid was used to make the amine group positively charged. The chip surface was rinsed with DI water and then treated in 0.25 mg/mL of GQDs aqueous solution in a polydimethylsiloxane (PDMS) stencil that had an array of eight wells in a diameter of 6 mm. The GQD-modified chip was kept in the dark overnight to ensure that the negatively charged GQDs were immobilized on the positive amine on the surface (GQDs-silanized glass). The GQD-silanized glass chip was rinsed with DI water, dried and kept at 4 °C for future use.

Functionalization of GQD-coated chips: A phosphate buffered saline (PBS) solution containing 40 mM 1-ethyl-3-dimethylaminopropyl carbodiimide (EDC) and 10 mM N-hydroxysuccinimide (NHS) was injected into individual wells and kept for 15 min. After washing with PBS buffer solution, 5 μg/mL CA-125 capture antibody (cAb) was added to each well and incubated at room temperature for 1 h in order to covalently link the cAb onto the GQDs, forming the GQDs~cAb chip. After rinsing with PBS, a blocking buffer solution containing 30 mg/mL bovine serum albumin (BSA) were added into each well and incubated for 1.5 h to prevent the non-specific adsorption of bio-molecules on the unoccupied sites. Finally, the GQDs~cAb chip surface was rinsed with the PBS buffer to remove all excessive and unreacted proteins.

Labeling detection antibody with horseradish peroxidase (HRP): 50 μL of 0.5 mg/mL HRP enzyme solution and 50 μL of 100 μg/mL detection antibody solution were mixed, followed by addition of 50 μL of 1 mg/mL NHS and 100 μL of 2 mg/mL EDC in PBS. After incubation with constant shaking at room temperature for 2 h, 5 μL of quenching buffer containing ethanolamine was added to stop the reaction. The resulting HRP-labeled detection antibody (Ab~HRP) was stored at 4 °C for 12 h and purified by centrifugation at 60,000 rpm for 30 min to separate and remove the unconjugated HRP from the supernatant.

Detection of CA-125 with the immunoassay A 30 μL of serial dilution of CA-125 antigen (0.1 U/mL to 600 U/mL) was prepared and applied in each well, and incubated for 1 h to allow the antigen to bind to the cAb immobilized on the glass chip, forming the GQDs~cAb+CA-125. The chip was then rinsed with PBS buffer. Next, the chip (GQDs~cAb+CA-125) was incubated in a PBS solution containing 2 μg/mL HRP-labeled detection antibody (Ab~HRP) at room temperature for 1 h. Subsequently, 0.1 M Tris buffer (pH 8.5) containing 0.2 mM H_2O_2 and 0.2 mM luminol was added. After 30 s, the chemiluminescence (CL) intensity at 460 nm from each well was measured using a fluorescence microplate reader (Synergy H4 Hybrid Reader, BioTek Instrument, Inc., Vermont). All the experiments were performed at room temperature.

To test the robustness of the assay in real-world samples, 20 μL of the immunoassay solution was spiked with 20 μL of blood plasma to prepare the sample matrix. CA-125 was then detected in such a sample matrix.

Characterization: The graphene quantum dots (GQDs) were characterized with a JEOL JEM 2100F transmission electron microscope (TEM, Japan) at an acceleration voltage of 200 kV. Fourier transform infrared (FT-IR) spectra was acquired using the transmission mode with a Thermo Nicolet 6700 spectrometer (Thermo Scientific, Waltham, MA) while the GQDs immobilized on the chip before and after antibody functionalization were characterized under the attenuated total reflection (ATR) mode. The X-ray photoelectron spectra (XPS) were measured with a PHI 5000 Versa Probe system (Physical Electronics, MN). The acquired spectra were calibrated with the C 1s as the reference peak of aliphatic carbon at 284.8 eV.

Figure S1 shows the nanoflake-shaped GQDs with the lateral size of around 40 nm. Figure S2 reveals the FT-IR spectrum of the GQDs powder. The FT-IR spectrum displayed several characteristic peaks including 1718 cm^{-1} (C=O in COOH), 1629 cm^{-1} (C=C in the skeletal ring), 1403 cm^{-1} (COO-), 1214 cm^{-1} (C-OH) and 1069 cm^{-1} (C-O-C) [S2,S3]. The FT-IR analysis has confirmed that there were abundant carbonyl, carboxylic acid, hydroxyl and epoxy moieties in the GQDs, which was in agreement with the previous result [S1]. The negatively charged -COOH group on the surface allowed the interaction with the $NH3^{+}$, which led to the immobilization of the GQDs on the silanized glass chips (Figure 1(b)). After attraction of the GQDs on the silanized glass slide, the characteristic FT-IR peaks were still present, which indicated the successful immobilization of the GQDs with the active functional groups (Figure S2). This can be further confirmed by the XPS analysis (Figure S3). The C 1s core-level XPS spectrum of the GQD-silanized glass in Figure S3 was deconvoluted into four components at 284.8 eV (C-C), 285.8 eV (C-O-C), 286.7 eV (C–OH or α-C in –C–COOH) and 289.3 eV (COOH) [S2,S3]. The N 1s XPS spectrum obtained from the GQDs-silanized glass can be deconvoluted into two components. The component at 402.1 eV was attributed to the protonation of the amine group [S4,S5], which came from the activated amine group on the silanized glass with the positive charge. The component at 400.5 eV was ascribed to N-C=O, which came from the week conjugation of amine on the glass with the -COOH moiety of the GQDs.

After functionalization of the GQD-silanized glass with the capture antibody as shown in Figure 1(c), the characteristic peaks at 1561 cm^{-1} and 1648 cm^{-1} for N-H and 1263 cm^{-1} for C-N [S7] appeared in the FT-IR spectrum while the peak at 1718 cm^{-1} disappeared (Figure S2). Consistently, the peak at 289.3 eV (COOH) disappeared in the C 1s XPS spectrum. Instead, a

peak at 288.6 eV (N-C=O) was observed (Figure S3). In addition, a strong N 1s peak at 399.6 eV showed up, which came from the C-N bonds in the antibody. Also, the intensity of peak at 400.5 eV (N-C=O) became much stronger due to the abundant peptide bonds in the antibody [S8]. In short, the FT-IR and XPS spectra confirmed the successful immobilization of GQDs and the functionalization of antibody on the glass chip.

References

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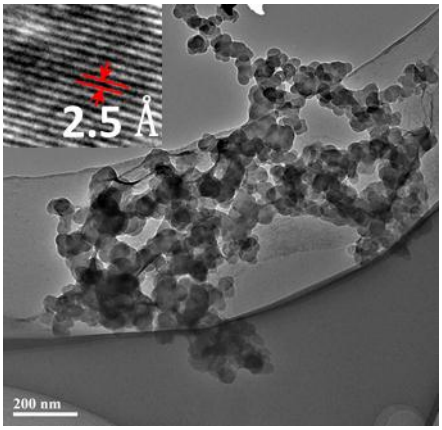

Figure S1 TEM image of the graphene quantum dots (GQDs).

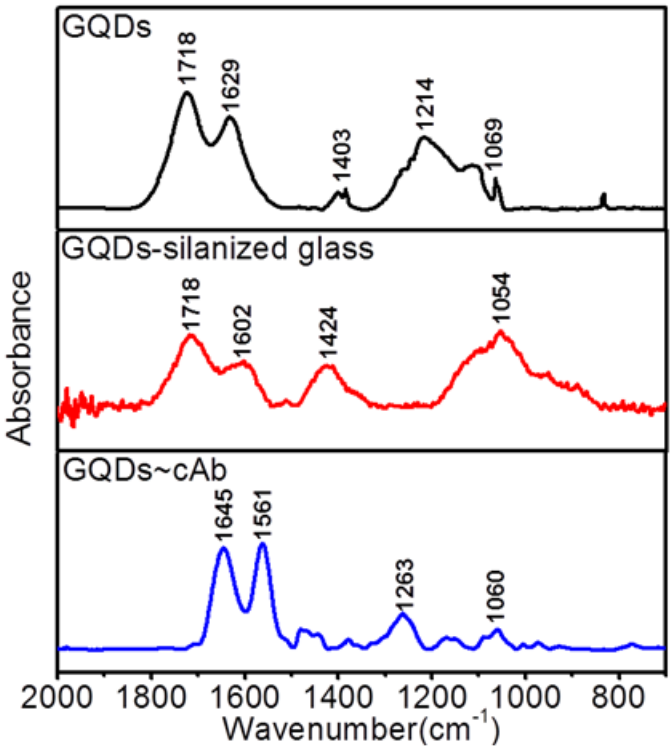

Figure S2 FTIR spectra of the free-standing GQDs, the GQDs immobilized on the silanized glass slide (GQDS-silanized glass), and the GQDs functionalized with CA-125 capture antibody (GQDs-cAb).

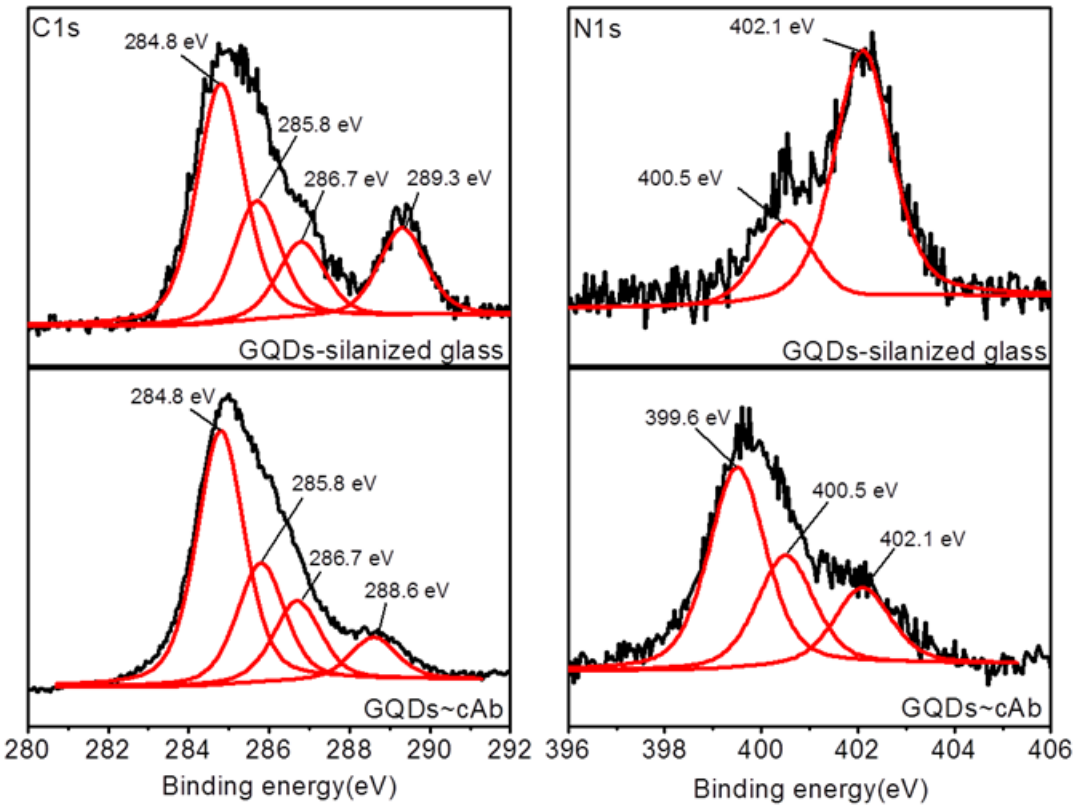

Figure S3 XPS spectra of the core-levels of C 1s and N 1s obtained from the GQDs immobilized on the silanized glass slide (GQDS-silanized glass), and the GQDs functionalized with CA-125 capture antibody (GQDs-cAb).

Optimizations of detection conditions

Tests were performed for optimization of the detection condition, including the pH, the ionic strength and the concentrations of both the capture antibody and the detection antibody. The best pH, which provided the highest chemiluminescence intensity for detection, was recorded at 8.5 as seen in Figure S4A. The capture antibody and the detection antibody concentrations were optimized to record the highest signal-to-noise ratio over a wide range antigen concentration. Thus, a high concentration of 600 U/mL antigens was selected to establish the best capture and detection antibody concentrations. The capture antibody concentrations at 1, 2, 5 and 10 μg/mL at a constant concentration of detection antibody (2 μg/mL) were tested. The results showed that 5 μg/mL of capture antibody was the best concentration to achieve the highest intensity. For the detection antibody, 2, 5, 25 and 50 μg/mL were tested at 5 μg/mL of the capture antibody in the presence of 600 U/mL of CA-125 antigen. The best signal-to-noise ratio was observed at 2 μg/mL of detection antibody, which yielded the lowest chemiluminescence intensity (Figure S4C). Moreover, to verify the effect of the ionic strength on the immunoassay, the NaCl concentration in the detection buffer was optimized by using various NaCl concentrations up to 0.3 M. The ionic strength has negligible effect on the sensing signal of immunoassay when the NaCl concentration changed from 0.075 M to 0.3 M.

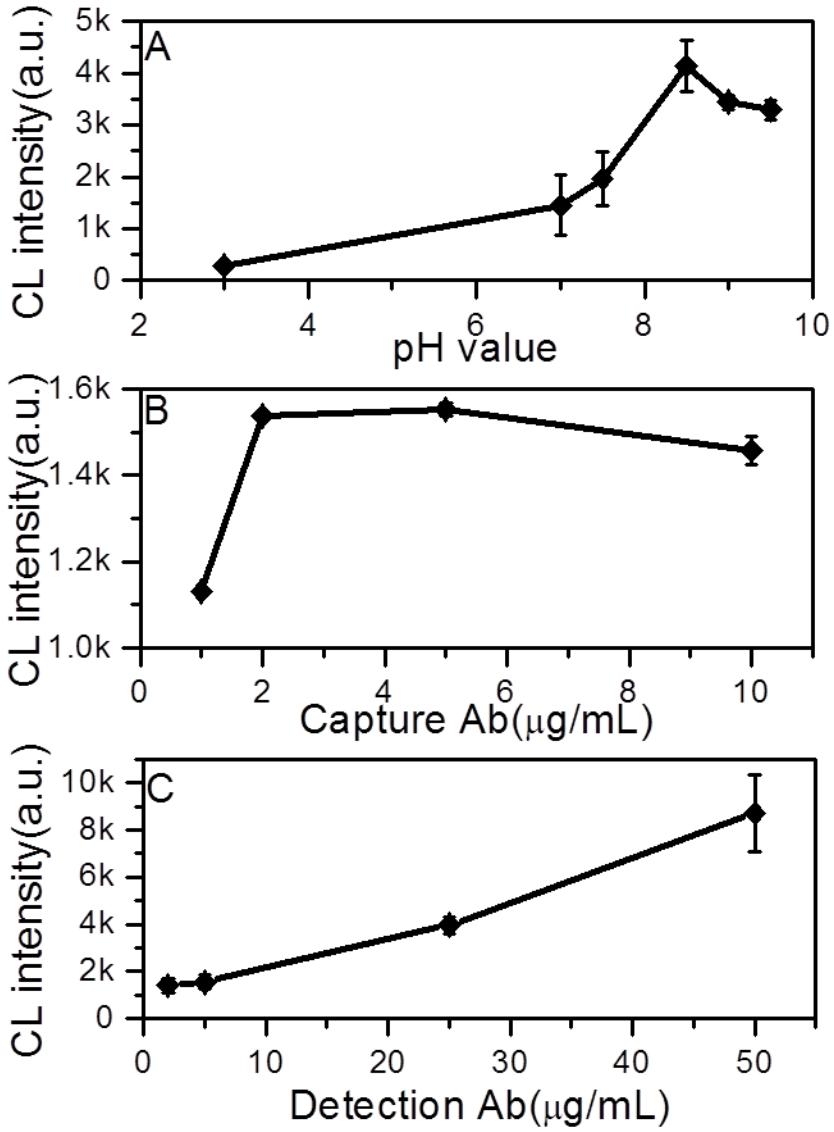

Figure S4 Optimization of the detection condition at different pH (A), different capture antibody (B) and detection antibody concentrations (C).

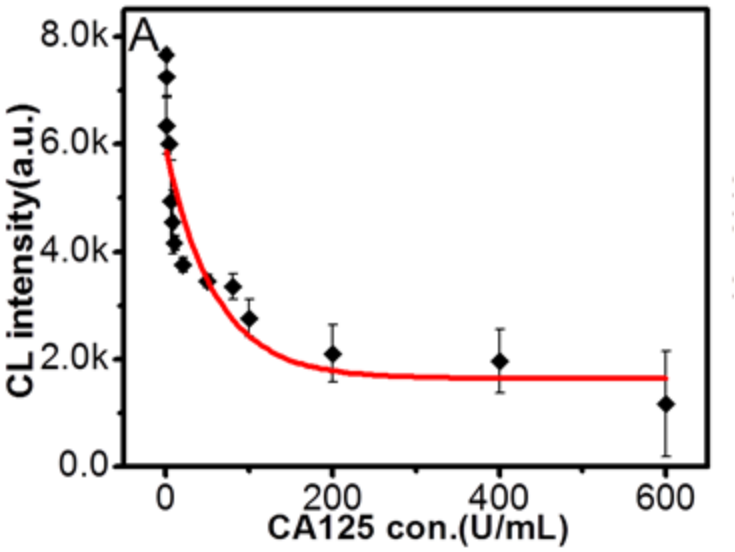

Figure S5 Chemiluminescence measured from the immunoassay containing various concentrations of CA-125 antigen

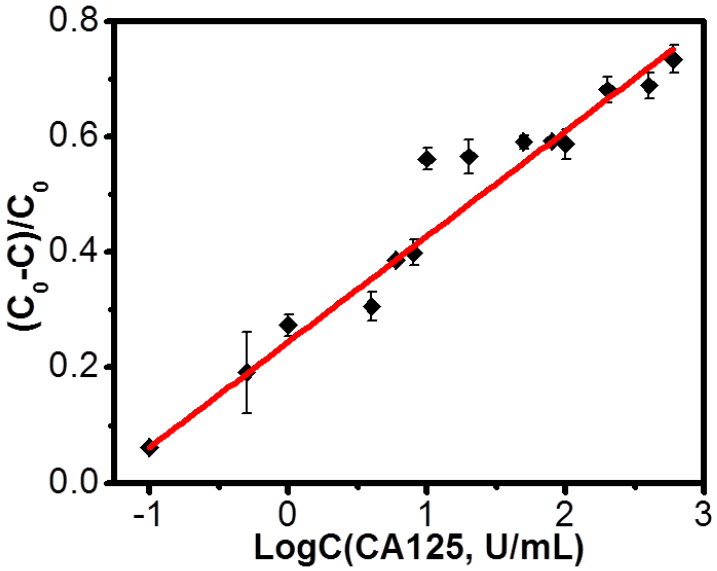

Figure S6 Linear correlation of the sensor response to the CA-125 concentration in the matrix containing 50% buffer and 50% blood plasma.