Supplementary Material for Chemical Communications

Supplementary data

A universal immunosensing strategy based on regulation of the interaction between graphene and graphene quantum dots†

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Instruments

The nanoscale-graphene sheets were characterized by transmission electron microscopy (TEM, FEI Tecnai G2 Spirit). Graphene quantum dots (GQDs) were characterized by atomic force microscopy (AFM, Agilent PicoPlus Ⅱ) and TEM. UV-Vis absorption spectra were recorded with a Hitachi UV-2450 spectrophotometer. Fluorescence (FL) and photoluminescence (PL) measurements were performed by using a Hitachi F-4500 spectrofluorimeter.

Synthesis

Materials and Reagents: Human immunoglobulin G (IgG), human immunoglobulin M (IgM), and monoclonal antibody mouse anti-human immunoglobulin G (mIgG) were purchased from Beijing Wanyumeilan Scientific Inc. Bovine serum albumin (BSA) was purchased from Beijing Aoboxing Bio-tech Co., Ltd. N-(3-dimethylaminopropyl)-N-ethylcarbodiimde hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were provided by J&K Chemical Ltd. All chemicals were used without further purification and ultrapure water (18.2 MΩ) was used through the whole experiment. The phosphate buffer solution (PBS, 20 mM, pH 8.0) was prepared by mixing the stock solution NaH_2PO_4 and Na_2HPO_4.

Preparation of Graphene (Gr)

Graphene oxide (GO) sheets were prepared from nature graphite powder by the modified Hummers method.[1] Briefly, 1 g graphite powder was added into 23 mL concentrated H_2SO_4 with vigorous stirring at 0 °C, and then 3 g $KMnO_4$ was slowly added under the same condition. After that, the mixing substance was sonicated for 6 h to give a dark green solution. Successively, 46 mL H_2O was transferred into the reaction system gradually. After 15 min, this reaction was terminated by 140 mL H_2O and 10 mL H_2O_2 (30%). Then the yellow resultant was

separated by centrifugation, and the sediment was washed by HCl (5%) and ultrapure water, respectively.

Gr was synthesized through a reported method.[2] Briefly, 50 mL GO aqueous solution (0.05 mg/mL) was transferred into a Teflon-lined autoclave and heated at 180 °C for 6 h. After autoclave cooling to the room temperature, the black homogeneous Gr solution was obtained.

Preparation of GQDs and GQDs with carboxyl group (GQDs-COOH)

GQDs were prepared on the basis of a reported method.[3] In short, 20 mg dried GO sheets and 2 mL N, N-dimethylformamide were added into 20 mL dichlorosulfoxide, and refluxed at 80 °C-85 °C for 72 h. The precipitate centrifugated was washed by anhydrous tetrahydrofuran and dried in vacuum. Then the residue and 1 mL n-butylamine were heated under nitrogen at 60 °C-65 °C for 96 h. The mixture was cooled down to the room temperature and dispersed in ultrapure water. At last, a light yellow-green supernatant (GQDs) was obtained after centrifugation.

A simple method was used to introduce carboxyl groups.[4] Briefly, 0.05 g NaOH and 0.1 g $ClCH_2COOH$ were added into 20 mL GQDs solution, followed by bath sonication for 3 h to covert hydroxyl groups into carboxyl groups. And the resultant product was neutralized with HCl.

Preparation of GQDs-mIgG bioconjugates

600 μL GQDs-COOH solution was mixed with 200 μL EDC (10 mg/mL) and 150 μL NHS (15 mg/mL). The mixture reacted for 20 min at room temperature. Then, 600 μL mIgG (1 mg/L) in PBS was added into the mixture. The samples were shaken slowly at 25 °C for 2 h in the dark and kept overnight at 4 °C to allow mIgG to covalently bind the GQDs surface. Then the product was centrifuged and filtrated through a filtration membrane (300 kDa) to remove unreacted substances, the residues on the membrane were dissolved in PBS (20 mM, pH 8.0) and stored at 4 °C.

Determination of human IgG

In a typical experiment, 60 μL Gr (0.05 mg/mL) was introduced into the resultant mIgG-GQDs/Gr solution at room temperature to initiate the quenching reaction. After 20 min reaction, the different concentrations of human IgG were added into the above mIgG-GQDs solution, and the mixture incubated at 37 °C for 1 h. Finally, the PL emission of GQDs was measured under 360 nm excitation wavelength.

Calculations

Quantum yield (QY) calculations:

When the absorbance was less than 0.05, the QY of GQDs was estimated using rhodamine B (ethanol as solvent, QY = 89%) as a reference standard by the following equation:

$$Q_x = Q_s(A_s S_x N_x^2)/(A_x S_s N_s^2)$$

Where Q represents the PLQX, A is the optical density at excitation wavelength that obtained from UV-Vis absorption spectra of CQDs, S is the integrated PL intensity, and N is the refractive index of solution. The subscript x and s denote the measured standard samples, respectively.

Figures

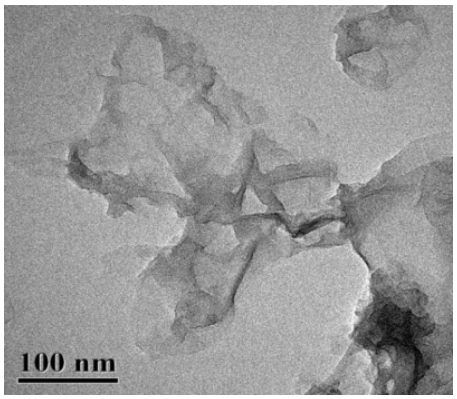

Fig. S1 TEM image of Gr. TEM samples were prepared by depositing a few drops of diluted Gr solution onto the cooper grids and dried naturally.

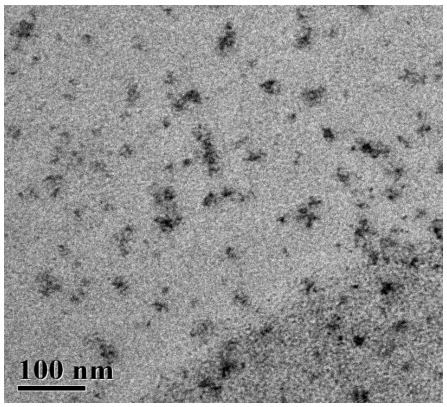

Fig. S2 TEM image of original GQDs. TEM samples were prepared by depositing a few drops of GQDs solution onto the cooper grids.

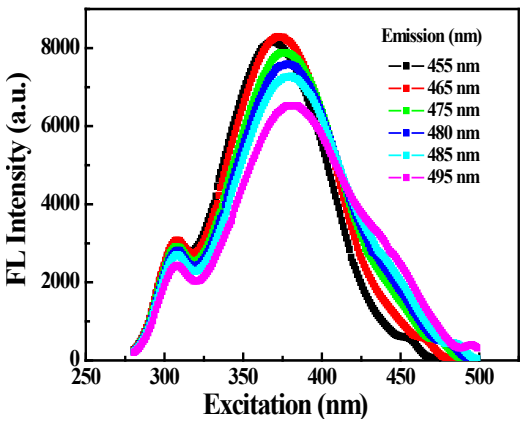

Fig. S3 The excitation spectra of GQDs under different emission wavelengths.

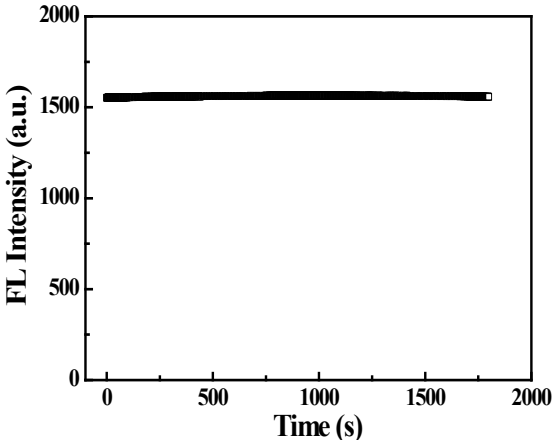

Fig. S4 Time-dependent FL change of GQDs under continuous illumination. λ_{ex} = 360 nm, λ_{em} = 460 nm.

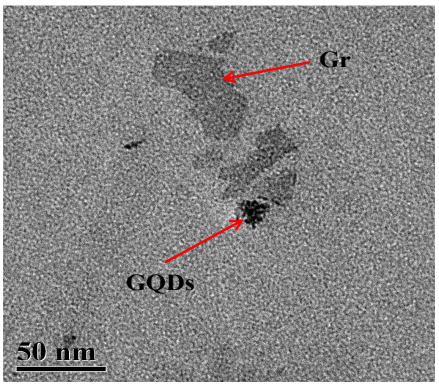

Fig. S5 TEM image of the mixture of GQDs and Gr.

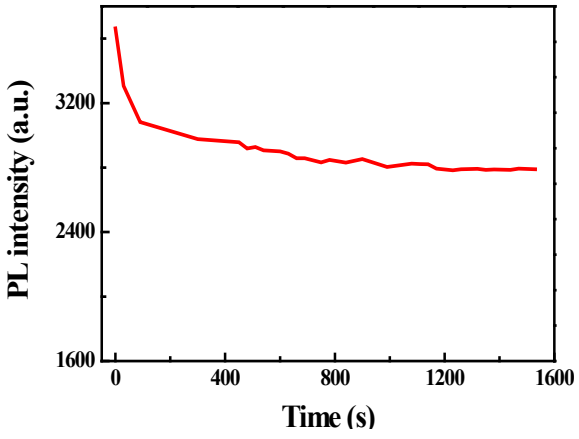

Fig. S6 Time-dependent PL change of mIgG-GQDs in the presence of 2 μg/mL Gr. λ_{ex} = 360 nm, λ_{em} = 460 nm.

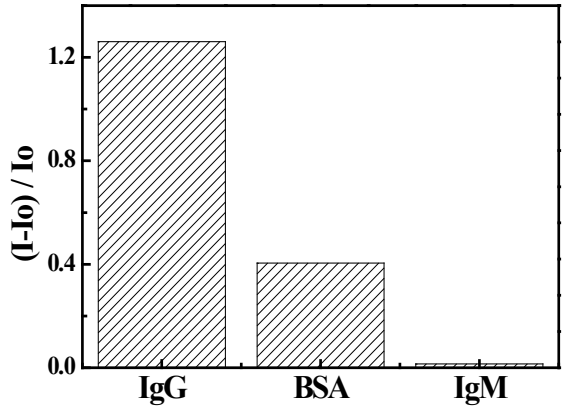

Fig. S7 Variations in PL intensity of mIgG-GQDs/Gr system induced by human IgG, BSA and human IgM, all with a concentration of 10 μg/mL. The PL intensity of mIgG-GQDs/Gr system was defined as I_0, and I was the PL intensity in the presence of antigens. λ_{ex} = 360 nm, λ_{em} = 460 nm.

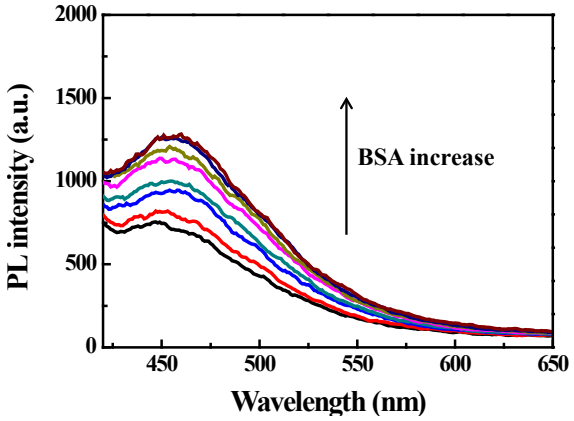

Fig. S8 Changes in the PL spectra of mIgG-GQDs upon increasing the concentration of BSA, with concentrations of 0, 1.25, 3.75, 5, 6.25, 7.5, 8.75, 10 μg/mL from bottom to top. λ_{ex} = 360 nm, λ_{em} = 460 nm.

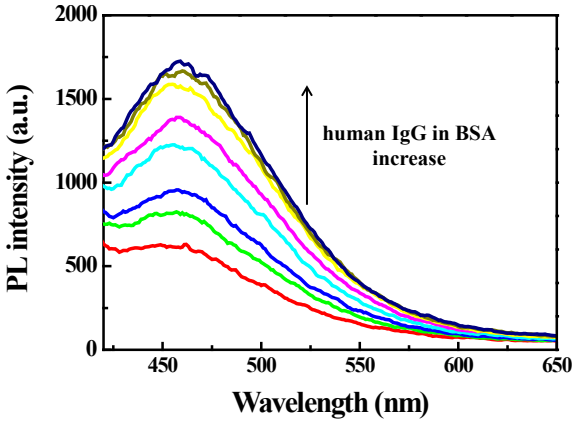

Fig. S9 Changes in the PL spectra of mIgG-GQDs upon increasing the concentration of human IgG in 10 μg/mL BSA, with concentrations of 0, 0.2, 2, 4, 10, 20, 30, 40 μg/mL from bottom to top. λ_{ex} = 360 nm, λ_{em} = 460 nm.

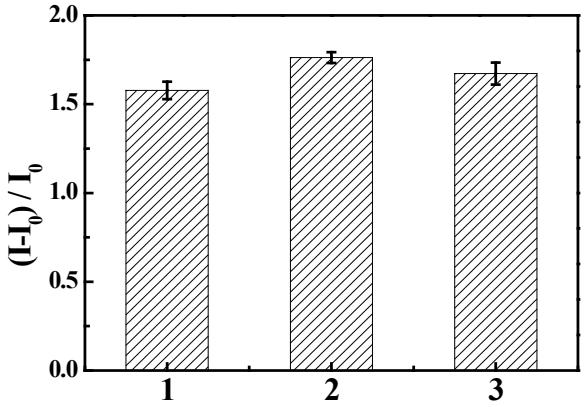

Fig. S10 Variations in PL intensity of mIgG-GQDs/Gr system induced by human IgG (1) 10 μg/mL in PBS, (2) 10 μg/mL in 25% human serum, (3) 10 μg/mL in 25% cell culture fluid. The PL intensity of mIgG-GQDs/Gr system was defined as I_0, and I was the PL intensity in the presence of antigens. λ_{ex} = 360 nm, λ_{em} = 460 nm.

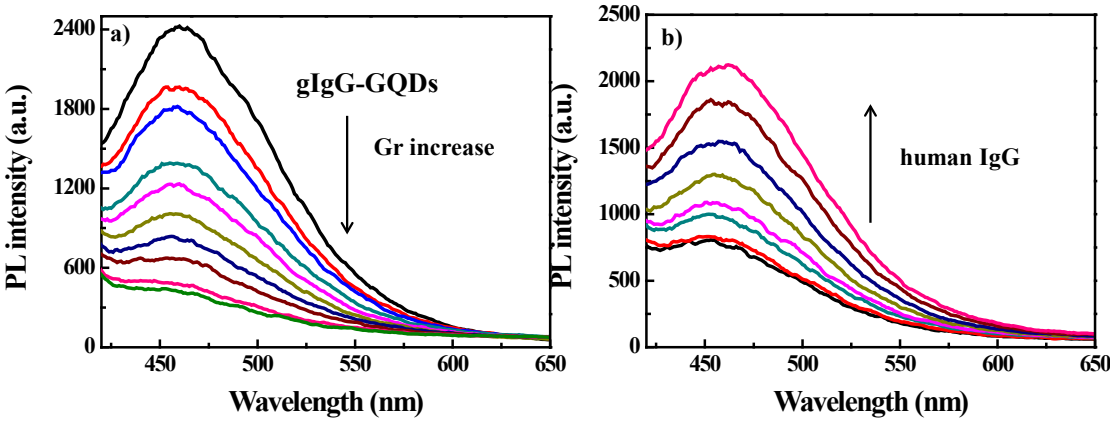

Fig. S11 a) Changes in the PL spectra of GQDs modified with goat anti-human immunoglobulin G (gIgG, antibody) in PBS (20 mM, pH 8.0) with an increasing concentration of Gr, with concentrations of 0, 1.25, 2.5, 2.6, 3.1, 3.75, 5, 6.25, 7.5, 8.75 μg/mL from top to bottom. b) Changes in the PL spectra of gIgG-GQDs upon increasing the concentration of human IgG, with concentrations of 0, 0.25, 2.5, 10, 12.5, 25, 37.5, 50 μg/mL from bottom to top. λ_{ex} = 360 nm, λ_{em} = 460 nm.

References

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(4) K. Yang, S. Zhang, G. Zhang, X. Sun, S.-T. Lee and Z. Liu, *Nano Lett.*, 2010, **10**, 3318-3323.