Electronic Supplementary Material (ESI) for Nanoscale

Supporting information

Self-assembled graphene quantum dots induced by cytochrome c: A novel biosensor for trypsin with remarkable fluorescence enhancement

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Experimental details

Reagents and materials: All reagents and chemicals were of analytical reagent grade and used without further purification. Cyt c was obtained from Aladdin Chemistry Co., Ltd. (Beijing, China). Trypsin, lysozym, papain, pepsin and bovine serum albumin were obtained from Beijing Dingguo Biotechnology Co., Ltd. (Beijing, China). Deionized water (18.2 MΩ·cm resistivity) from a Milli-Q water system was used throughout the experiments. Protein solutions were stored at 4℃ before use. GQDS were used as the FL probes in this work. All experiments were performed in a PBS buffer (20 mM phosphate, pH 8.86).

GQDS were synthesized as follows according to our previously published reports.[1] 50 mg GO was dispersed in 10 mL DMF. The GO/DMF solutions were under ultrasonication for 30 minutes (120 W, 100 kHz), and then transferred to a poly (tetrafluoroethylene) (Teflon)-lined autoclave (30 mL) and heated at 200 ºC for 8 h. After the above reaction, the reactors were cooled to room temperature by water or naturally. The product contained brown transparent suspension and black precipitates, and the black precipitates were wasted. The solid samples can be obtained by evaporating the solvents. Then, the GQDs were purified by column chromatography on silica utilizing gradient elution (mobile phase: A was Methylene Chloride-MeOH (2:1, V/V), B was H_2O). Under the A phase elution, the obtained Batch 1 and 2 were wasted. Subsequently, under the B phase elution, Batch 3 was obtained, the Batch 3 is the primary production with a yield of ca. 50-70% which is as used GQDs in this manuscript.

Apparatus: FL profiles were obtained with 5301PC luminescence spectrometer (Shimadzu, Japan), and the record the fluorescence spectra from 345 to 600 nm with an excitation wavelength at 320 nm. Quartz cuvettes with 1-mm pathlength were used for emission measurements. Atomic force microscopy (AFM) images were recorded using atomic force microscopy (SPA-300, Seiko Instruments, Japan). A droplet of GQDs and the GQDs-Cyt c complex dispersion (0.6 mg/mL) was cast onto a freshly cleaved mica surface, followed by drying at room temperature. The microscopic features of GQDs and GQDs-Cyt c complex dispersion (0.6 mg/mL) were characterized using a JEM-1011 TEM (JEOL, Tokyo, Japan) with a CCD cinema. UV-vis absorption spectra were recorded on a Lambda-800 spectrophotometer.

FL spectra of GQDs in the presence of different amounts of Cyt c: variable amounts of Cyt c stock solutions (8.0 mM) were mixed with 25 μL buffer (PBS, c=200 mM, PH=8.86) in eppendorf cups, respectively. Then 5 μL GQDs (3 mg/mL) were mixed with the samples. Subsequently, water was added to eppendorf cups to ensure the total volume of the reaction mixture was 250 μL and the emission spectra were recorded at room temperature.

FL response of GQDs-Cyt c complex in the presence of different amounts of trypsin: Different concentrations of trypsin was first added into the eppendorf cups containing GQDs (60 μg/ml) and cyt c (1.0 mg/ml) in 20 mM PBS buffer at pH 8.86, The eppendorf cups were then incubated at 37 °C over 24 h. The FL spectra of the mixtures were measured under excitation at 320 nm. The emission spectra were recorded at room temperature.

FL response of GQDs-Cyt c complex towards control proteins: 600 μg/ml of bovine

serum albumin, lysozym, papain, pepsin, trypsin was added into the eppendorf cups containing GQDs (60 μg/ml) and cyt c (1.0 mg/ml) in 20 mM PBS buffer at pH 8.86, respectively. Then the mixtures were incubated at 37 °C over 24 h. The FL spectra of the mixtures were measured under excitation at 320 nm at room temperature.

FL response of GQDs towards arginine and lysine: 25 μL arginine stock solutions (8.0 mM) and lysine stock solutions (8.0 mM)were mixed with 25 μL buffer (PBS, c=200 mM, PH=8.86) in eppendorf cups, respectively. Then 5 μL GQDs (3 mg/ml) were mixed with the samples. Subsequently, water was added to eppendorf cups to ensure the total volume of the reaction mixture was 250 μL. Then the mixtures were incubated at 37 °C over 24 h and the FL spectra of the mixtures were measured at room temperature.

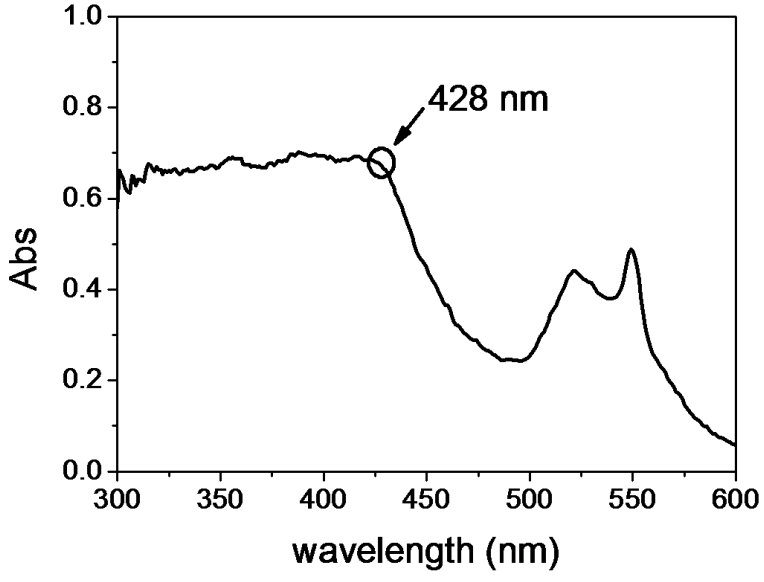

Fig. S1 Absorption spectrum of Cyt c (1000 μg/mL) in PBS buffer (20 mM, pH 8.86).

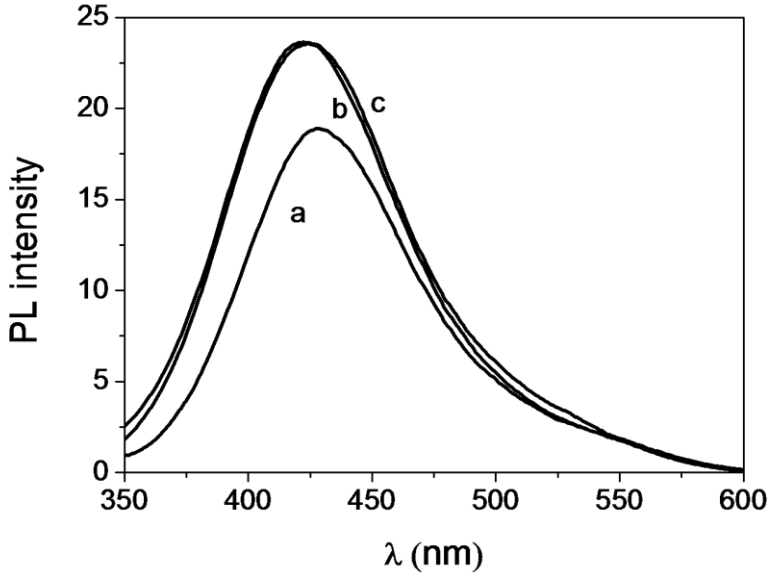

Fig. S2 FL emission spectra of GQDs (60 μg/ml) at different conditions: (a) GQDs in PBS buffer; (b) GQDs + Arginine (1000 μg/mL); (3) GQDs + Lysine (1000 μg/mL).

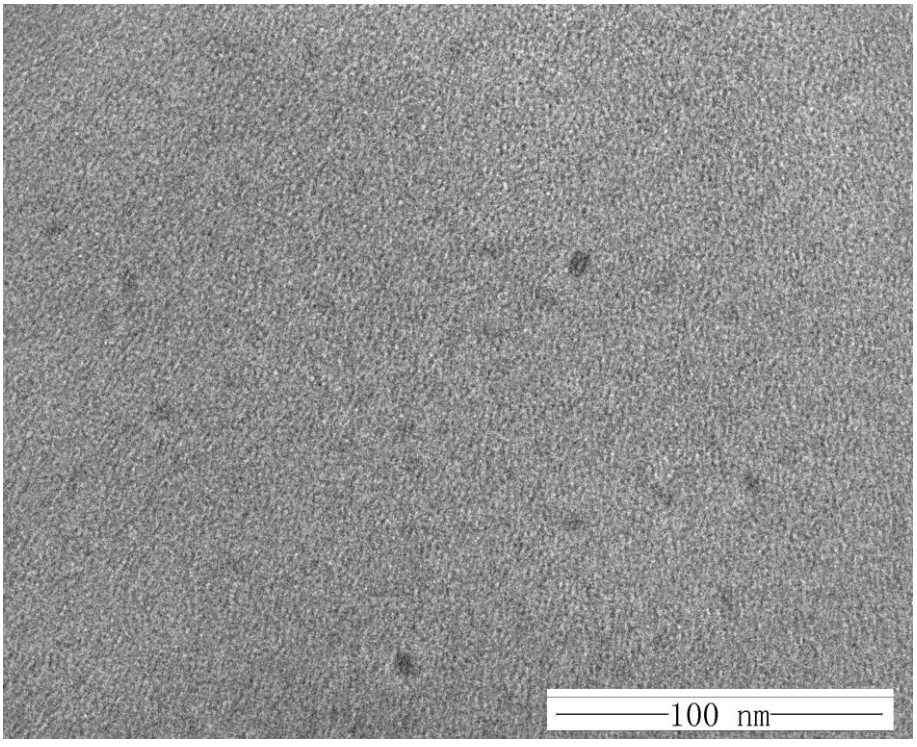

Fig. S3 TEM images of the self-assembled GQDs induced by Cyt c fragments.

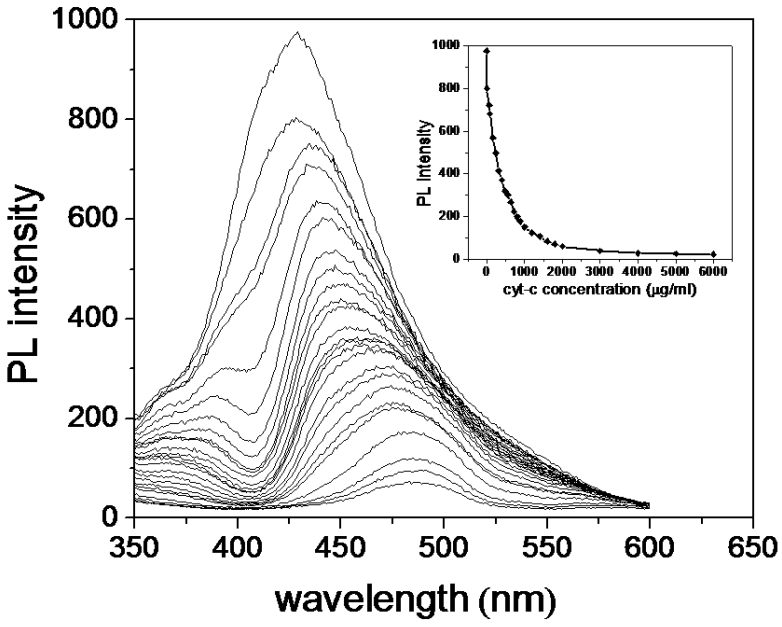

Fig. S4 Fluorescent emission spectra of GQDs (60 μg/ml) in the absence of (top curve) and presence of the Cyt c (the other curves, Cyt c concentration from 0 μg/ml to 6000 μg/ml) in PBS buffer (20 mM, pH 8.86). The inset shows a plot of changes in FL intensity at 428 nm against changes in Cyt c concentration.

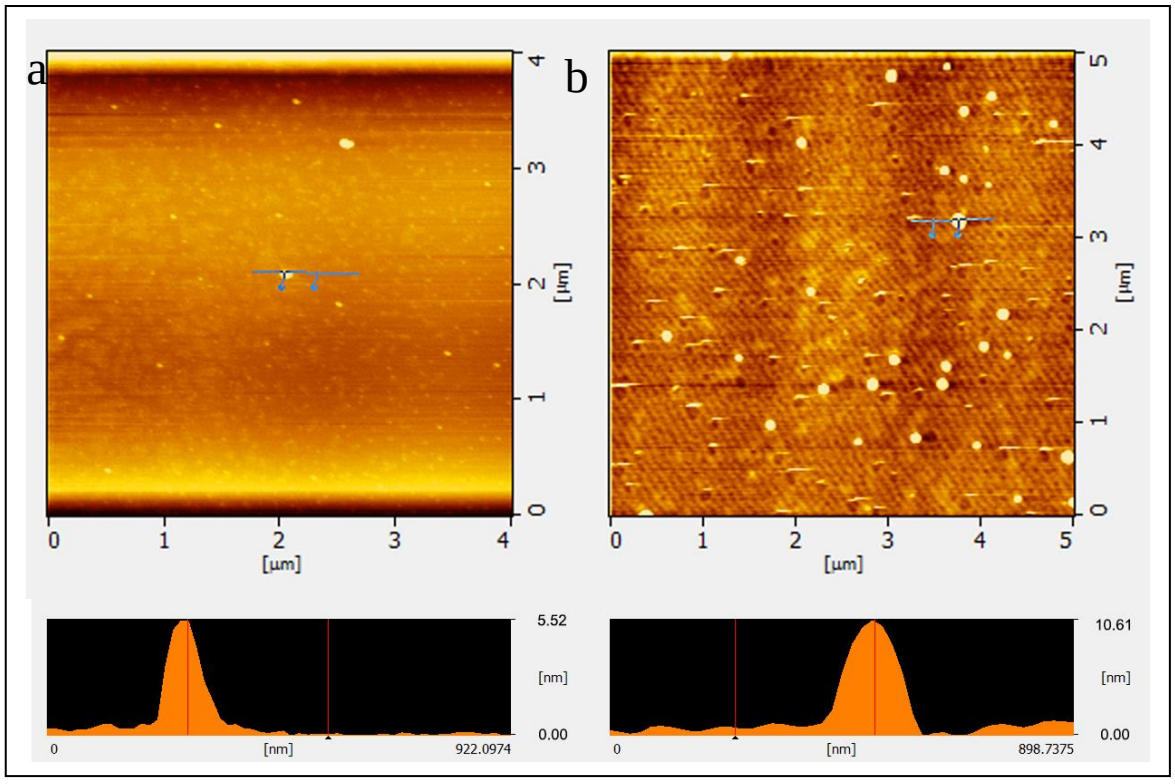

Fig. S5 AFM images of the GQDs (a) and their self-assembled aggregates by Cyt c (b).

1. S. Zhu, J. Zhang, C. Qiao, S. Tang, Y. Li, W. Yuan, B. Li, L. Tian, F. Liu, R. Hu, H. Gao, H. Wei, H. Zhang, H. Sun and B. Yang, *Chem. Commun.* , 2011, **47**, 6858; S. Zhu, J. Zhang, X. Liu, B. Li, X. Wang, S. Tang, Q. Meng, Y. Li, C. Shi, R. Hu and B. Yang, *Rsc Advances*, 2012, **2**, 2717.