Supplementary Material for Chemical Communications

Supplementary data

Distance-Independent Quenching of Quantum Dots by Nanoscale-Graphene in Self-Assembled Sandwich Immunoassay

Meng Liu, Huimin Zhao, Xie Quan,* Shuo Chen and Xinfei Fan

Instruments

The nanoscale-graphene nanosheets were characterized by atomic force microscopy (AFM, Agilent PicoPlus□). UV-Vis absorption spectra were recorded with a Hitachi UV-2450 spectrophotometer. FL measurements were performed using a Hitachi F-4500 spectrofluorimeter. Time-resolved PL measurements were performed by FluoroMax-4-TCSPC spectrometer (HORIBA Jobin Yvon). The FT-IR measurements on powdered samples were made using a Bruker VERTEX 70 Fourier Transform Infrared Spectrometer in transmission mode with a KBr window. All measurements were performed at ambient conditions. Purified bioconjugates were analyzed by sodium dodecyl Sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The acrylamide concentrations for the concentrating and separating gels were 4% and 8%, respectively. The samples were run at 120 V for 90 min and were imaged using a G: BOX HR system (Gene Co., Ltd).

Synthesis

Materials and Reagents: α-fetoprotein (AFP), monoclonal antibodies to AFP (Ab1, Ab2 and Ab5) and bovine serum albumin (BSA) were provided by Beijing Wanyumeilan Scientific Inc. N-(3-dimethylaminopropyl)-N-ethylcarbodiimde hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from J&K Chemical Ltd. All other reagents were of analytical reagent grade and used without further purification. Ultrapure water (18.2 MΩ) was used throughout the experiments. The 0.02 M phosphate buffer solution with pH 8.0 was prepared by mixing the stock solution of K_2HPO_4 and KH_2PO_4.

Preparation of Graphene oxide (GO) and Hydrothermal Converted Graphene (HCG)

GO was synthesized from graphite powder by a modified Hummers method.[1] Briefly, graphite powder (1.2 g) was ground with NaCl (50 g) for about 20 min. NaCl was then removed by filtration and the resultant ground graphite were washed several times with ultrapure water. After dried at 50 °C, the pretreated graphite powder was dispersed in concentrated sulfuric acid (23 mL) at room temperature, and potassium permanganate ($KMnO_4$, 3 g) was slowly added with vigorous stirring at 0 °C. The mixture was then sonicated for 6 h to give a dark green solution. Successively, 46 mL distilled water was gradually transferred to the reaction system. The reaction was maintained for 10 min, and then terminated by distilled water (140 mL) and H_2O_2 solution (30%, 10 mL). Then the resultant yellow product was separated by centrifugation, and washed with 5% HCl solution and ultrapure water, respectively. HCG was prepared by a reported method.[2] The obtained aqueous dispersion of HCG (0.5 mg/mL) was stored at room temperature and employed in the following experiments.

Preparation of sulfonated GO and HCG

A simple sulfonation method was used to introduce sulfonic acid groups.[3] Briefly, 50 mg sulfanilic acid and 19.8 mg

sodium nitrite were firstly dissolved in 10 mL NaOH solution (0.25%), and then added to 10 mL 0.1 M HCl in ice bath under stirring. After reaction for 10 min, the aryl diazonium salt solution was added to 50 mL 0.5 mg/mL GO dispersion and was kept stirring for 2 h in ice bath. After filtration and rinsing, the purified sulfonated GO was diluted to 0.1 mg/mL and stored at 4 °C. The sulfonated HCG was prepared by the same method.

Preparation of QDs-Ab5, QDs-Ab2, GO-Ab1 and HCG-Ab1 bioconjugates

The procedure was similar to previous reports using EDC and NHS as coupling reagents.[4] Typically, 200 µL CdTe QDs (in 20 mM phosphate buffer, pH 8.0) were firstly activated with 20 µL EDC (10 mg/mL) and 15 µL NHS (15 mg/mL). The mixture was gently mixed for 20 min at 25 °C. Then 60 µL Ab1 (Ab2) (2.275 mg/L) in phosphate buffer was added to the mixture. The samples were shaken slowly at room temperature for 2 h in the dark and kept overnight at 4 °C. To remove the free nonconjugated complex, the above mixture was then centrifuged at 13000 rpm for 30 min at 4 °C. After that, the sediment was washed and redispersed in phosphate buffer solution. In order to reduce the non-special interaction, 2% BSA solution was added. For the preparation of GO-Ab1 and HCG-Ab1 bioconjugates, 400 µL GO (0.1 mg/mL) and HCG (0.1 mg/mL) were activated with 10 µL EDC (5 mg/mL) and 20 µL NHS (3 mg/mL), respectively. And then exposed to 15 µL Ab1 (12 mg/mL) in 20 mM phosphate buffer (pH 8.0) overnight at room temperature. The nonconjugated complex was removed by centrifugation and filtration (MWCO 100 kDa filter), and the resultant product was stored at 4 °C.

Estimation of QDs-HCG center-surface distance

Monoclonal antibodies-Ab5 and Ab1 including two antigen-binding fragments and one crystalline fragment were ~ 80 Å long in three-dimensional structure. According to its molecular weight of 68 kDa, the minimum size of "V"-like AFP was ~ 50 Å. For CdTe QDs emitting at 507 nm, the radius was ~ 13 Å. A resultant distance $d = d_{Ab5}+d_{Ab1}+r_{QD}+d_{AFP}$ = 80 Å +80 Å +13 Å +50Å = 223 Å was calculated.

Figures

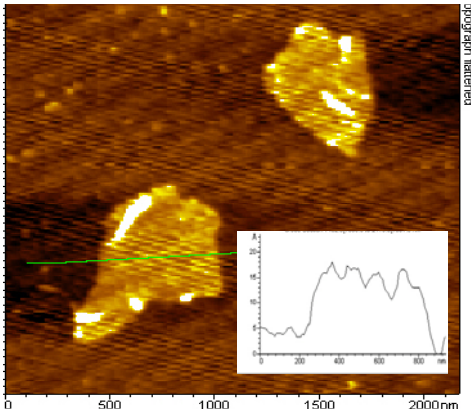

Fig. S1. AFM image of as-prepared HCG sheets on freshly cleaved mica.

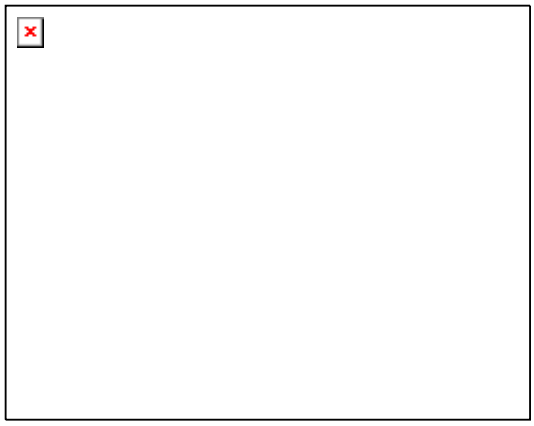

Fig. S2. FTIR spectra of the GO, sulfonated-GO, HCG and sulfonated-HCG measured in KBr pellets.

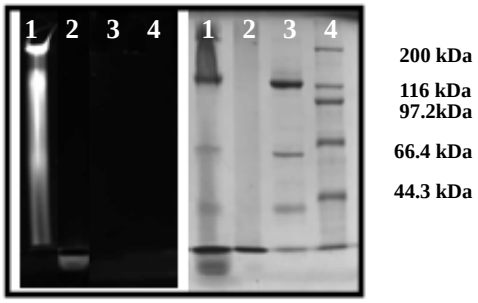

Fig. S3. Left panel: SDS-PAGE luminescence image under UV illumination, right panel: SDS-PAGE image stained by Coomassie Blue. Lane 1: Ab5-QDs; lane 2: QDs; lane 3: Ab5; lane 4: standard protein marker.

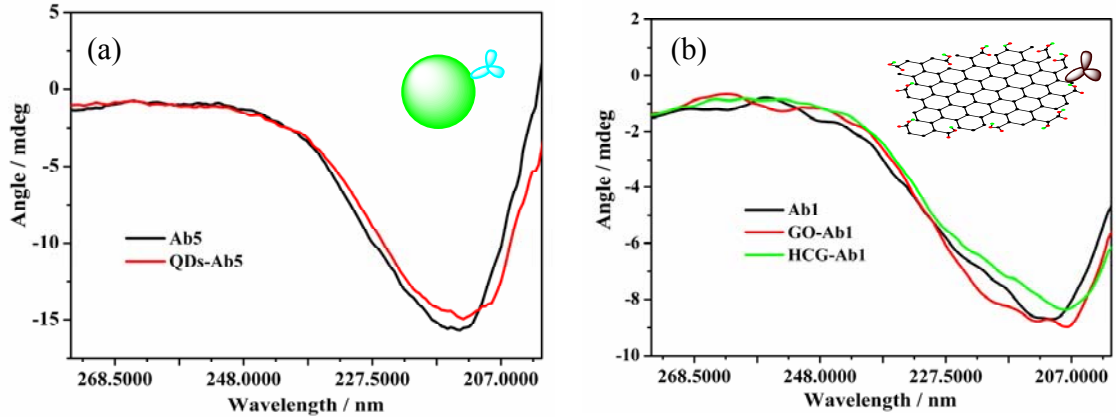

Fig. S4. Circular dichroism spectra of Ab5 and Ab1 before (a) and after (b) their conjugation to QDs and HCG sheets, respectively. Samples were recorded in 20 mM phosphate buffer (pH 8.0).

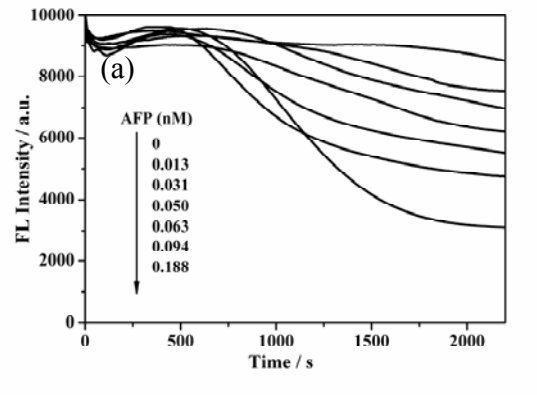

Figure S5. (a) Time-dependent FL changes of our system in the presence of various AFP concentrations. Fixed concentration: [Ab5-QDs] = 12.78 nM, [Ab1-HCG] = 7.5 nM. Scan time: 2200 s, delay time: 0 s, response time: 8 s. (b) The relationship between the concentration and FL intensity for subsystem 1: Ab5-QDs/AFP/Ab1-HCG, and subsystem 2: Ab5-QDs/AFP/Ab1-GO. The error bars represent the standard deviation. (c) Normalized time-resolved FL decay of CdTe QDs in different subsystems and free in HCG (GO) solution without the addition of AFP. Fixed concentration: [AFP] = 0.188 nM.

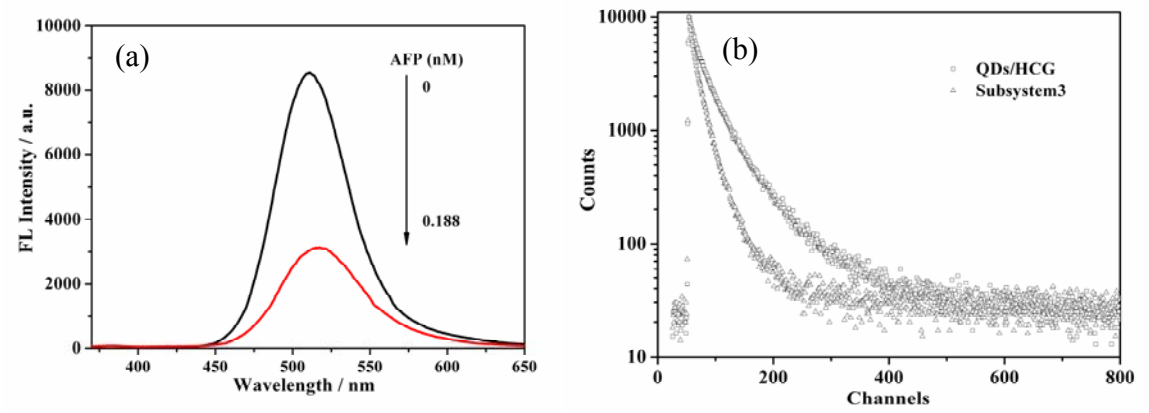

Fig. S6. (a) Emission spectra of the subsystem 3: Ab2-QDs/AFP/Ab1-HCG. (b) Normalized time-resolved FL decay of CdTe QDs in subsystem 3 and free in HCG (GO) solution without the addition of AFP. Fixed concentration: [Ab2-QDs] = 12.78 nM, [Ab1-HCG] = 7.5 nM, [AFP] = 0.188 nM, λ_{ex} = 340 nm, λ_{em} = 507 nm.

Table S1. FL lifetime measurements of QDs

system	B1	τ1 (ns)	B2	τ2 (ns)	B3	τ3 (ns)	<τ> (ns)	CHISQ	α	η
Ab5-QDs/HCG	0.253	10.076	0.360	37.382	0.0395	105.0	47.85	1.225		
Ab5-QDs/GO	0.200	9.439	0.349	38.623	0.0367	104.9	49.01	1.337		
Ab2-QDs/HCG	0.241	7.776	0.367	30.753	0.0519	85.02	41.94	1.306		
Subsystem1	0.201	11.633	0.026	46.164	0.9099	1.751	16.56	1.382	0.989±0.05	0.34±0.04
Subsystem2	0.189	3.839	0.485	22.641	0.0419	67.696	30.50	1.309	0.965±0.03	0.58±0.03
Subsystem3	0.305	3.619	0.427	16.514	0.0301	48.856	19.98	1.412	0.869±0.02	0.36±0.02

B1, τ1, B2, τ2, B3, τ3 are the amplitude values and corresponding lifetime for the three-exponential fitting components, respectively, <τ> is the averaged lifetime value and CHISQ is the χ^2 goodness of fit, η=1−E was derived from the steady-state FL loss, α was a ratio that the change in radiative rate in the presence of HCG or GO. Subsystem 1: Ab5-QDs/AFP/Ab1-HCG, Subsystem 2: Ab5-QDs/AFP/Ab1-GO, Subsystem 3: Ab2-QDs/AFP/Ab1-HCG.

References

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(2) Y. Zhou, Q. Bao, L. L.Tang, Y. Zhong and K. P. Loh, *Chem. Mater.*, 2009, **21**, 2950-2956.

(3) Y. Si and E. T. Samulski, *Nano Lett.*, 2008, **8**, 1679-1682.

(4) S. P. Wang, N. Mamedova, N. A. Kotov, W. Chen and J. Studer, *Nano Lett.*, 2002, **2**, 817-822.