Bio-conjugation of graphene quantum dots for targeting imaging

Materials.

EDC (e7750) were purchased from Sigma. Sulfo-NHS (24510) was purchased from Thermo Scientific.EZ-Link™ Sulfo-NHS-LC-Biotin. Streptavidin (016-000-084) was purchased from Jackson Immunoresearch. Anti mouse IgG was purchased from VECTOR.

GQDs synthesis was based on the reported method.[1]

300 mg graphite powder was dispersed in mixed acid (containing concentrated HNO_3 20 mL and concentrated H_2SO_4 60 mL).[1, 2] Then put the solution into a 100 mL round-bottom flask, stirred at 120 °C for 10 h. After the reaction, dilute the solution by pouring it into 300 mL di-water, followed by neutralizing the acid with Na_2CO_3. Put the solution into the refrigerator to remove the Na_2SO_4 salt from the solution as much as possible (repeat three times). Aggregation in the solution was then excluded by filter membrane of 220 nm. Finally, 3500 dialysis bag was used to further purify the sample.

The GQDs were further modified by the reported dimethyl formamidesolvothermal method.[3, 4] 9 mL DMF was added to a 1 mL CD aqueous solution (0.1–20 mg/mL) and this was subjected to ultrasonication for 10 min. After that, the mixed solution was put into a 30 mL Teflon-lined autoclave, and heated at 200 °C for 5 hours. The obtained product was collected by rotary evaporation. The GQDs were further 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, less bright GQDs were obtained. Subsequently, under the B phase elution, GQDs with strong brightness was obtained.

GQDs was conjugated with streptavidin (SA).

5 μL GQDs solution (10 mg/mL) was added 5 μL EDC (0.1 M) and 5 μL s-NHS (0.1 M), then 50 μL 1*MES was added. After shaker for 20 min, NHS ester modified GQDs was washed by Amicon centrifugal filter for 4 times using PBS solution (This step is used for removing excess EDC and s-NHS). After that, 11 μL SA (100 μM) was added and another 50 μL PBS was added. The reaction system was stirred at 4 ℃ refrigerator for 12 hours. The final product was purified by density gradient ultracentrifuge (DGU) with sucrose gradient from 15, 20, 25, 30, 35, 40, 45, 50 %. The volume of the DGU tube is 4 mL, and the volume of each sucrose fraction is 450 μL, after tilting the tube at 30°for 45 min, the sample was added at the top of the tube. Final, the DGU tubes were ultra-centrifuged for 10 hours at 31 k rpm. The final concentration of the SA@GQDs was determined by the UV-vis.

IR-680 conjugated with anti mouse IgG.

Take 90 uL 2 mg/mL (ca. 10 uM) anti mouse, add 10 uL 2 mM IR-680-NHS/DMSO solution, mixed well and incubate at RT in dark for 1.5 h. The final product was purified by 30 k Amicon centrifugal filter for 4 times using PBS solution, and the final concentration of Anti mouse@IR-680 was determined by UV-vis.

Bovine Serum Albumin conjugated with biotin (BSA-biotin).

6 mg BSA was dissolved in 1 mL 1*PBS, at the same time, 1 mg EZ-Link™ Sulfo-NHS-LC-Biotin was dissolved in 180 μL 1*PBS. Add the EZ-Link™ Sulfo-NHS-LC-Biotin to BSA to react at room temperature for 1.5 hour (shaker). After reaction, the volume was concentrated to 100 μL by 30 k Amicon centrifugal filter. At last, the BSA-biotin was purified by illustra NAP-5 columns (GE Healthcare, 17-0853-01). The final concentration of BSA is 4 mg/mL.

Cell imaging.

1. SK-OV-3 cells were cultured in McCoy's 5A medium plus10% FBS. The cell line was maintained in a 37 °C incubator with 5% (v/v) CO_2 for 24 h firstly. Cells were then incubated with100 nM mouse anti-HER2 in 5% BSA for 2 h. Cells were then washed three times with PBS, further incubated with 200 nM anti-mouse@GQDs, added into the cells container and incubated for 2 h. At last, the cells were labeled with DAPI (5 μg/mL) for 30 min, and were washed with PBS and scanned by EVOS FL.
2. SCC cells were cultured in DMEM medium plus 10% FBS. The cell line was maintained in a 37 °C incubator with 5% (v/v) CO_2 for 24 h firstly. Cells were then incubated with 200 nM Erbitux@GQDs, added into the cells container and incubated for 2 h. At last, the cells were labeled with DAPI (5 μg/mL) for 30 min, and were washed with PBS and scanned by EVOS FL.

Tissue staining.

1.1 Bring the tissue slides.
1.2 Fix in 4% PFAfor 20 min. Wash sections 3 times.
1.3 Treatment for 20 min with 0.1% Triton and 0.3% H_2O_2 in TBST.
1.4 Wash with TBST 3 times.
1.5 Blocking with 10% goat serum for 1 hour.
1.6 Apply CD31 and mouse anti-Her 2 (200 nM for each in 10% goat serum), 200 uL for each slice. Incubate overnight at 4 °C refrigerator.
1.7 Wash tissue using TBST 3 times.
1.8 Apply SA@GQDs (200 nM) mixed with Anti mouse@680 (20 nM), 200 ul for each slice. 1 hour at room temp.
1.9 Add Dapi 100 uL for each slice.
1.10 Wash tissue 3 times with TBST (5 min for every time) and scanned by fluorescence microcopy.

Characterization.

Fluorescence spectroscopy was performed with a Shimadzu RF-5301 PC spectrophotometer. UV-vis absorption spectra were obtained using a Shimadzu 3100 UV-vis spectrophotometer. Cell and tissue imaging were placed on fluorescence imaging system. Gel electrophoresisis done by commercial Bolt™ 4-12% Bis-TrisPlus Gels. All the bioimaging and staining picture was analyzed by Image J.

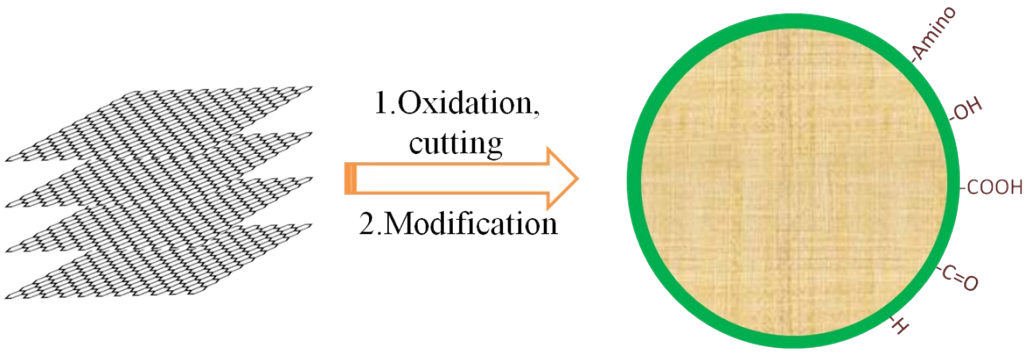

Figure S1. Scheme of preparing GQDs by oxidizing acid based "nano-cutting" and further modification method.

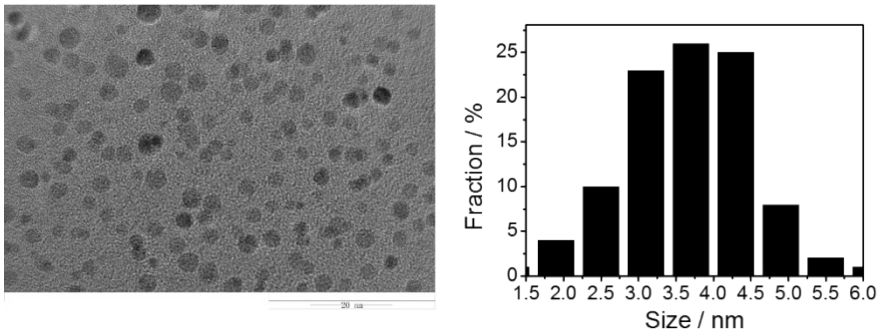

Figure S2. TEM image and size distribution of GQDs.

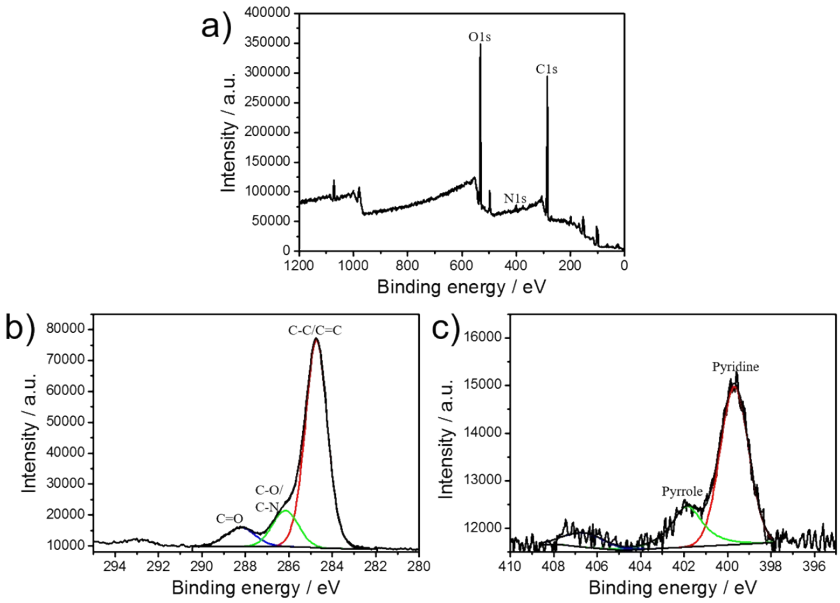

Figure S3. XPS analysis of GQDs with whole survey, C1s and N1s spectra.

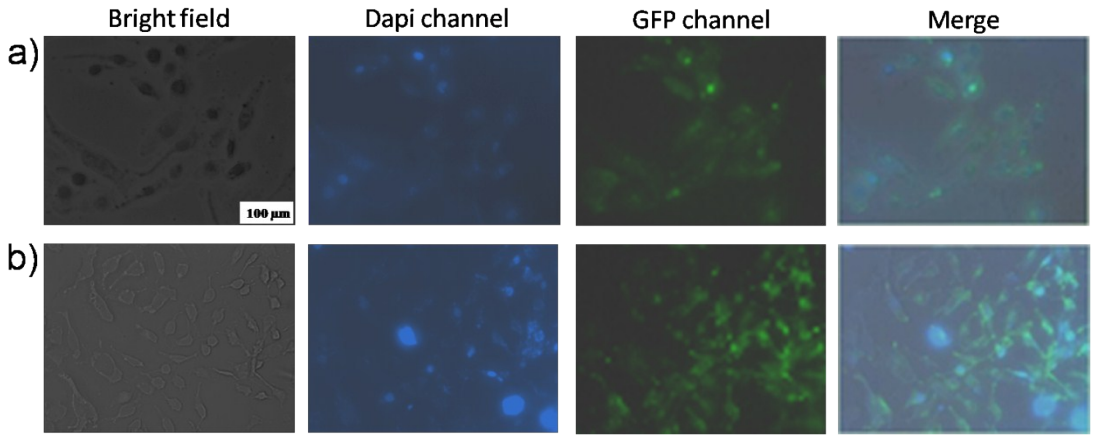

Figure S4. Random selected areas of SKOV 3 cell imaging (staining by anti mouse@GQDs and Dapi).

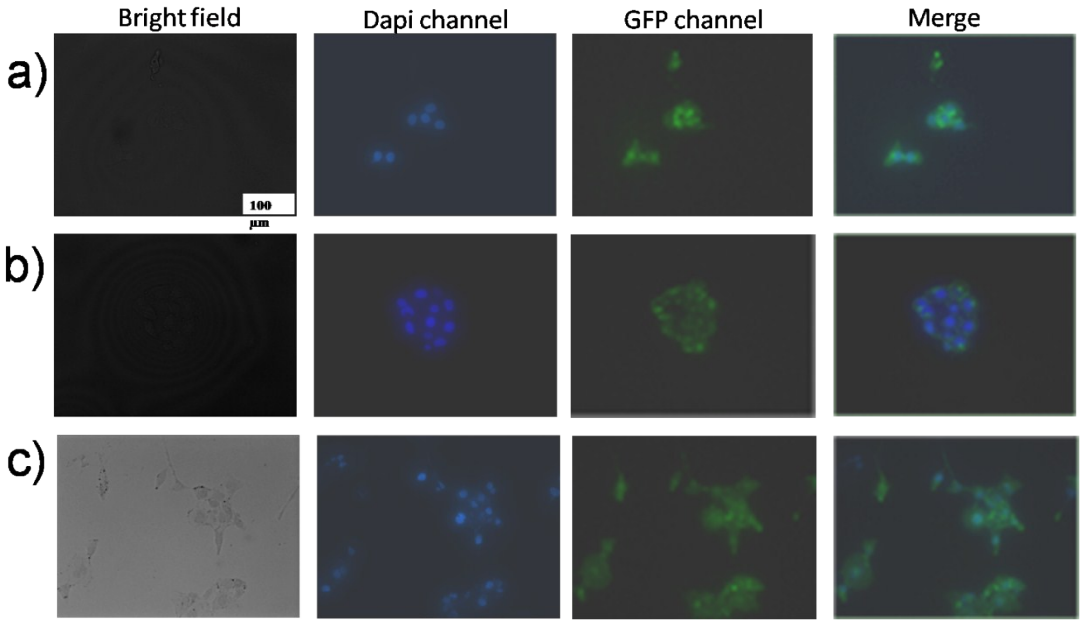

Figure S5. Random selected areas of SCC cell imaging (staining by Erbitux@GQDs and Dapi).

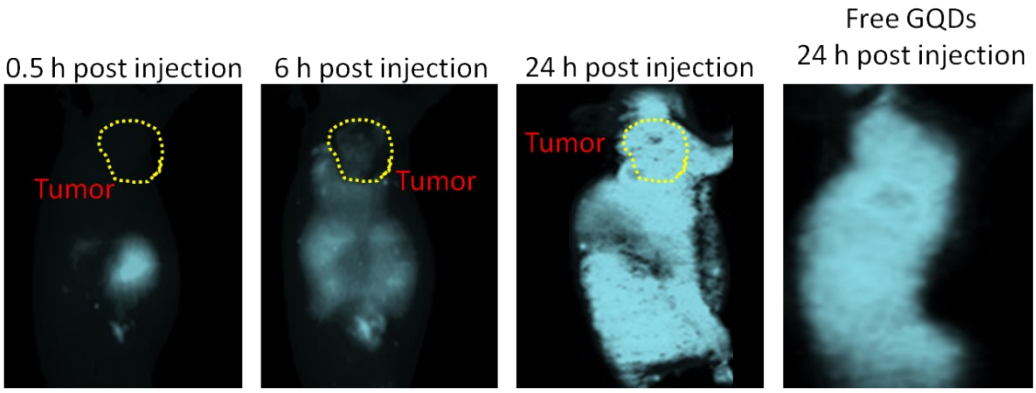

Figure S6. In vivo SCC tumor targeting imaging of Erbitux@GQDs and free GQDs.

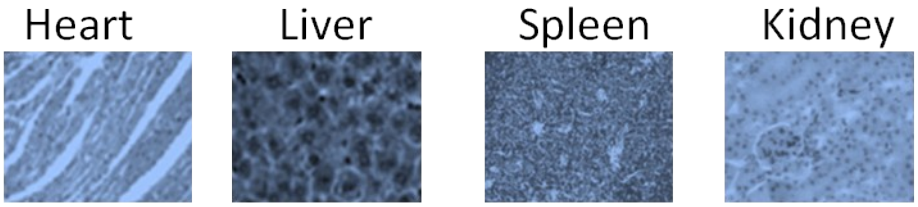

Figure S7. H&E staining study of GQDs@conjugate shows very low toxicity of the present conjugate.

References

1. S. Zhu, J. Shao, Y. Song, X. Zhao, J. Du, L. Wang, H. Wang, K. Zhang, J. Zhang and B. Yang, *Nanoscale*, 2015, **7**, 7927-7933.
2. S. Zhu, N. Zhou, Z. Hao, S. Maharjan, X. Zhao, Y. Song, B. Sun, K. Zhang, J. Zhang, H. Sun, L. Lu and B. Yang, *RSC Adv.*, 2015, **5**, 39399-39403.
3. S. Zhu, J. Zhang, S. Tang, C. Qiao, L. Wang, H. Wang, X. Liu, B. Li, Y. Li, W. Yu, X. Wang, H. Sun and B. Yang, *Advanced Functional Materials*, 2012, **22**, 4732-4740.
4. 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.