Supporting Information

Hybrid Gold Nanocube@Silica@Graphene-Quantum-Dot Superstructures: Synthesis and Specific Cell Surface Protein Imaging Applications

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1. Experimental section

1.1 Chemicals

$HAuCl_4 \cdot 4H_2O$, CTAB and ethanol were purchased from Shanghai Chemical Factory (Shanghai, China) and used as received without further purification. N-Hydroxysuccinimide (NHS), ethyl(dimethylaminopropyl) carbodiimide (EDC), 3-Aminopropyltrimethoxysilane (APTMS), 3-mercaptopropyltrimethoxysilane (MPTMS), and $NaBH_4$ were obtained from Aldrich. Anti-Epidermal Growth Factor Receptor antibody produced in goat and Epidermal Growth Factor Receptor human (buffered aqueous glycerol solution, 5,000-30,000 units/mg protein) were obtained from sigma. Graphite was purchased from Alfa Aesar. Other chemicals were of analytical grade and used without further purification. Water used throughout all experiments was purified with the Millipore system.

1.2 Synthesis and characterization of the hybrid AuNCs@SiO_2@GQDs nanostructures

Gold nanocubes preparation: Gold nanocubes were grown following a method report previously [6a]. Specifically, the seeds were prepared by the addition of a freshly prepared, ice-cold aqueous $NaBH_4$ solution (0.01 M, 0.6 mL) into an aqueous mixture solution composed of $HAuCl_4$ (0.01 M, 0.25 mL) and CTAB (0.1 M, 7.5 mL), followed by rapid inversion mixing for 2 min. The resultant seed solution was kept at room temperature for 1 h before use. The growth solution was prepared by the sequential addition of CTAB (0.1 M, 6.4 mL), $HAuCl_4$ (0.01 M, 0.8 mL), and ascorbic acid (0.1 M, 3.8 mL) into water (32 mL). The CTAB-stabilized seed solution was diluted 10 times with water. The diluted seed solution (0.02 mL) was then added into the growth solution. The resultant mixture solution was mixed by gentle inversion for 10 s and then left undisturbed overnight.

GQD preparation: GQDs were grown following a method report previously with little modification 3d. GO sheets were prepared from natural graphite powder by Hummers method. GO sheets (0.05 g) were oxidized in concentrated H_2SO_4 (10 mL) and HNO_3 (30 mL) for 15–20 h under mild ultrasonication (500 W, 40 kHz). The mixture was then diluted with deionized water and filtered with a nylon membrane (0.22 μm) to remove the acids. Purified oxidized GOs (0.2 g) were re-dispersed in DI water (40 mL) and the pH was tuned to 8 with NaOH. The suspension was transferred to a poly(tetrafluoroethylene) (Teflon)-lined autoclave (50 mL) and heated at 200 ^{0}C for 10 h. After cooling to room temperature, the resulting black suspension was filtered through a nylon membrane (0.22 μm) and a brown filter solution was separated. The colloidal solution was

further dialyzed in a dialysis bag (retained molecular weight: 3500 Da) overnight.

AuNCs@SiO_2@GQDs preparation: AuNCs@SiO_2@GQDs nanocomposites were synthesized according to a modified procedure from ref. 3g. 3-mercaptopropyltrimethoxysilane (MPTMS) was used for activation the gold nanocube surfaces before the silica shell deposition. Then 3-aminopropyltrimethoxysilane (APTMS) was used to modify the silica surface. The mixture was heated to 60 °C for 24 hours and then amine-functionalized AuNCs@SiO_2 nanocomposites were collected by centrifugation, washed twice with ethanol, and dispersed in deionized water. The GQDs was mixed with the suspension of amine-functionalized AuNCs@SiO_2 nanocomposites under mild stirring for at least 2 hours. The GQDs were self-assembled onto AuNCs@SiO_2 nanocomposites surfaces due to the interaction of the amine groups and the QDs. Finally, the hybrid AuNCs@SiO_2@GQDs nanocomposites were collected by centrifugation, washed twice with hexane, re-dispersed in water.

Anti-EGFR functionalized AuNCs@SiO_2@GQDs nanocomposites preparation: AuNCs@SiO_2@GQDs nanocomposites were dispersed in PBS (20 nM) to yield a stable suspension. Then 1mL AuNCs@SiO_2@GQDs nanocomposites were added with a 500 μL solution of anti-EGFR (1 mM) in 0.1m HEPES buffer (pH 7.5) in the presence of EDC (0.1 M) and NHS (0.1 M). The composites were allowed to equilibrate 2h with constant stirring. The Anti-EGFR functionalized AuNCs@SiO_2@GQDs nanocomposites were collected by centrifugation, followed by washing with PBS three times.

1.3 Cell culture

The Hela cell line were provided by the Institute of Biochemistry and Cell Biology (China). Cells were grown in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FBS (Fetal Bovine Serum) in an atmosphere of 5% CO_2 and 95% air at 37°C.

1.4 In Vitro Confocal Fluorescence Microscopy Imaging.

HeLa cells were grown on coverslips for 24 h, and then anti-EGFR functionalized AuNCs@SiO_2@GQDs nanocomposites were added with a final concentration of 0.05 nM. After incubation at 37°C and in 5% CO_2 atmosphere for 2 h, cells were washed with PBS three times before analysis to remove unassociated nanoparticles and then added with the fresh culture medium. These cell samples were then ready for imaging measurements. All confocal images were collected with a Leica inverted epifluorescence/reflectance laser scanning confocal microscope.

1.5 Apparatus

TEM images were obtained with a JEM-2100F high-resolution transmission electron microscope operating at 200 kV. UV-vis absorption spectra were recorded on a Cary 500 UV-vis spectrophotometer (Varian, U.S.A.). The FL spectra were recorded by a Perkin-Elmer LS55 Luminescence Spectrometer (Perkin-Elmer Instruments U.K.) using a 1-cm path length quartz cell at room temperature. The slot widths of the excitation and emission both were set at 10.0 nm. Infrared spectra were collected in transmission mode on a Nicolet 520 FTIR spectrometer.

2. The XPS data for AuNCs@SiO_2@GQDs nanocomposites

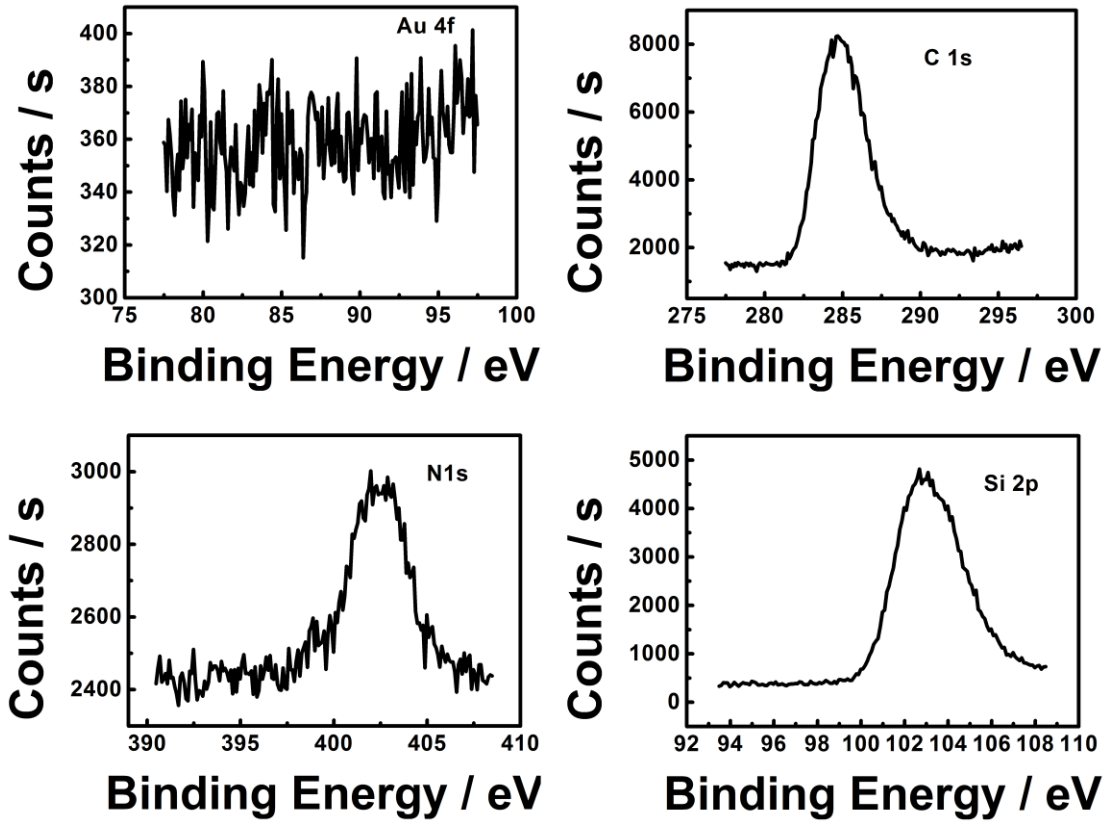


Figure S1 XPS Au 4f (A), C 1s (B), N 1s (C) and Si 2p (D) spectra of obtained AuNCs@SiO_2@GQDs nanocomposites

3. UV-vis absorption spectra of gold nanocubes

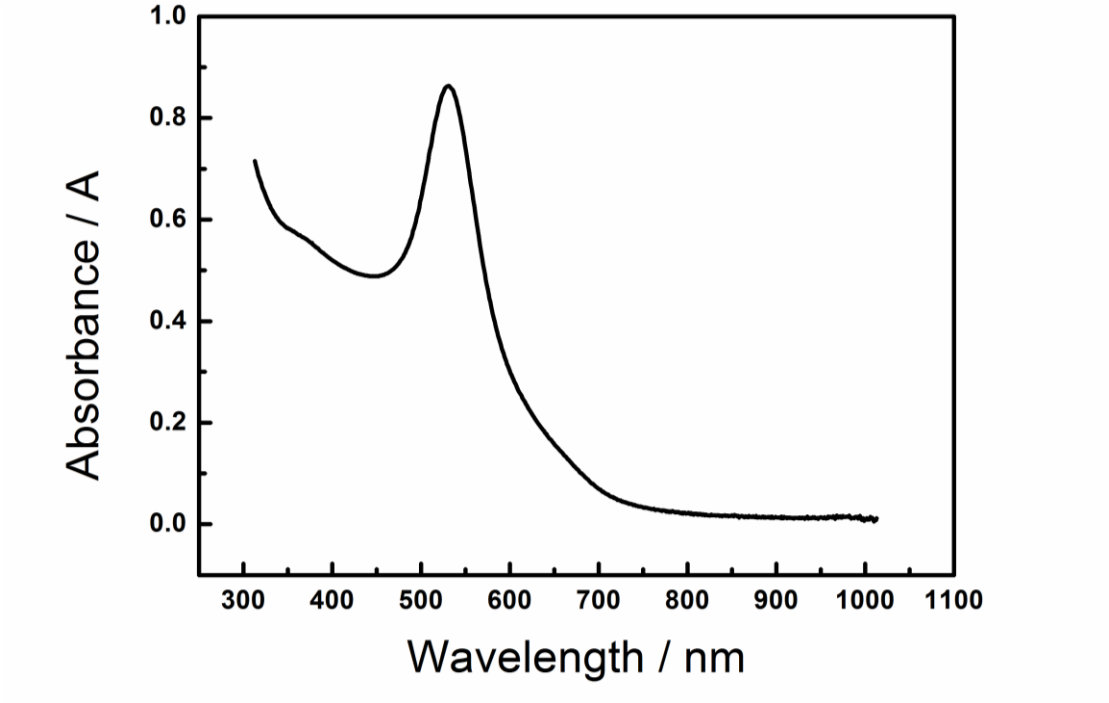


Figure S2 UV-vis spectra of obtained gold nanocubes.

4. UV-vis absorption spectra of AuNCs@SiO_2

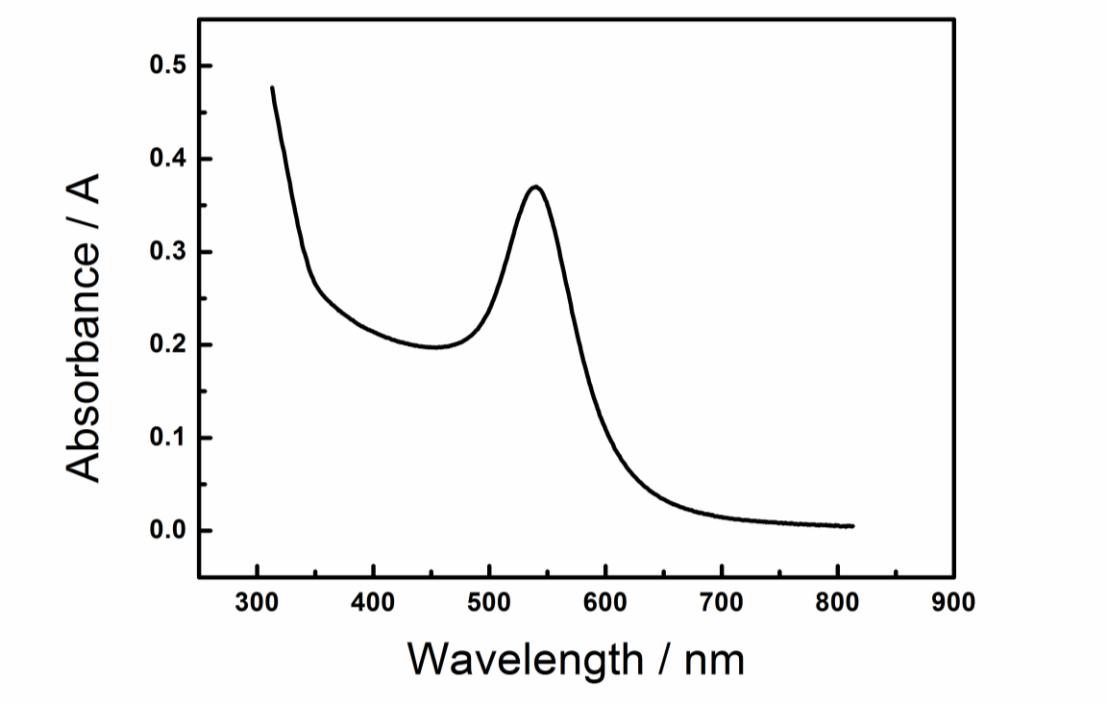
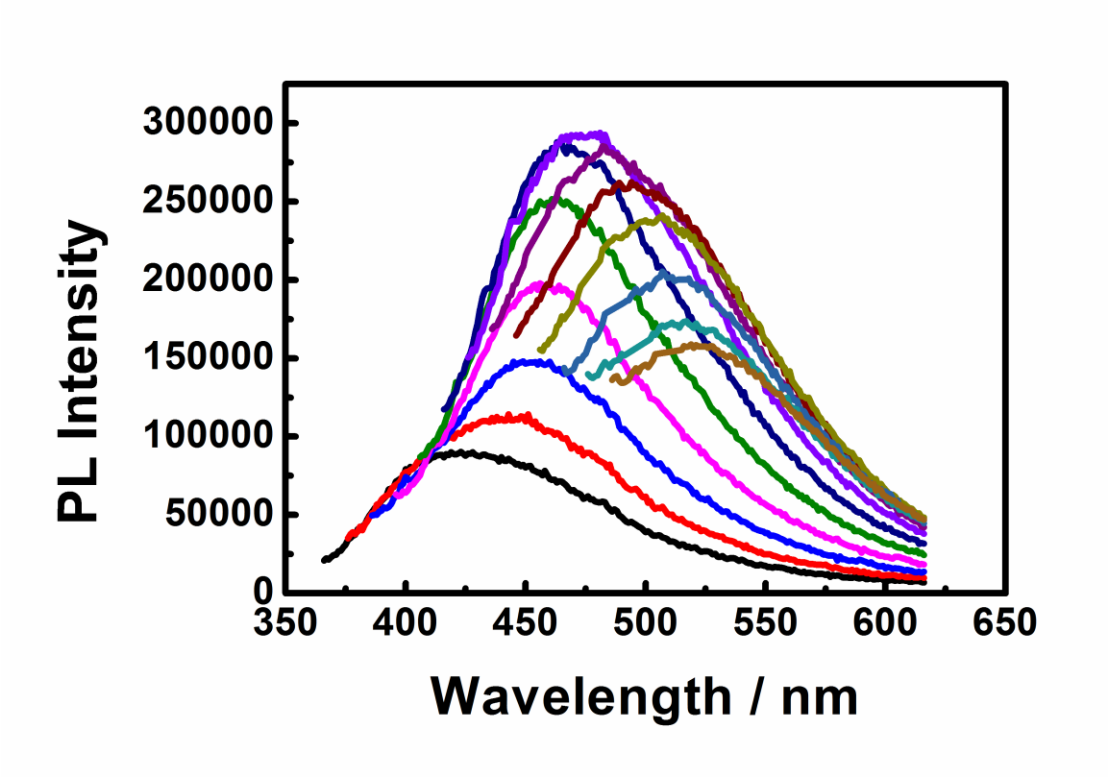


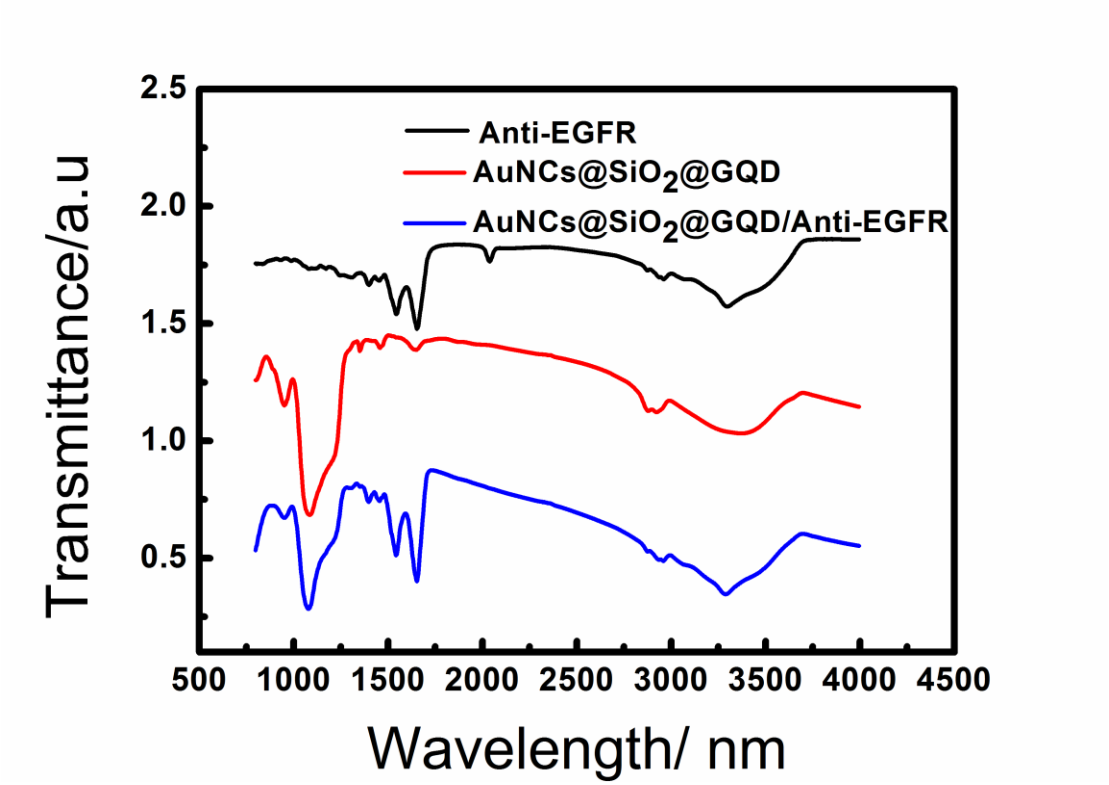
Figure S3 UV-vis spectra of prepared AuNCs@SiO_2.

5. PL spectra of the GQDs at different excitation wavelengths



Fiugre S4 PL spectra of the GQDs at different excitation wavelengths.

6. The FT-IR spectrum of anti-EGFR functionalized AuNCs@SiO_2@GQDs



Fiugre S5 The FT-IR spectrum of EGFR (black line), AuNCs@SiO_2@GQDs (red line), and anti-EGFR functionalized AuNCs@SiO_2@GQDs (blue line).

7. The Confocal fluorescent images of HeLa cells incubated with AuNCs@SiO_2@GQDs

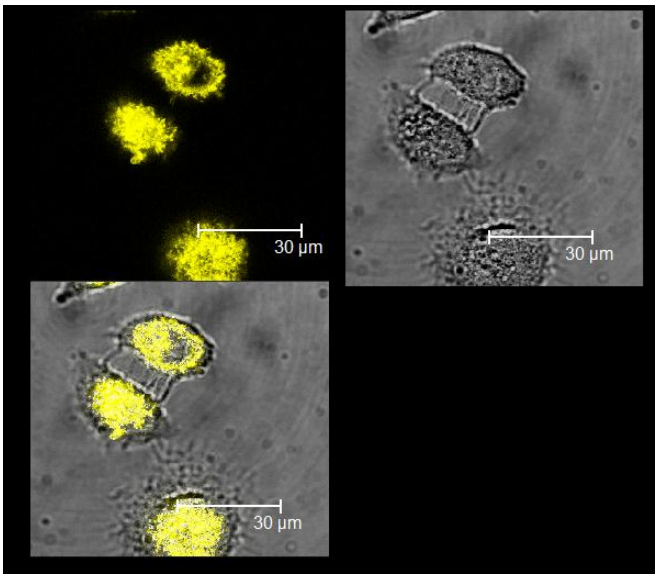


Figure S6 Confocal fluorescent images of HeLa cells incubated with AuNCs@SiO2@GQDs nanocomposites with concentrations of 0.05 nM for 2 h.