Electronic Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Aqueous one-pot synthesis of bright and ultrasmall CdTe/CdS near-infrared-emitting quantum dots and their application for tumor targeting *in vivo*

Li-Na Chen,[‡a] Jing Wang,[‡a] Wen-Tao Li[b] and He-You Han*[a]

[a]*State Key Laboratory of Agricultural Microbiology, College of Science, Huazhong Agricultural University, Wuhan 430070, China. Fax: +86 27 87288246; Tel.: +86 27 87288246; E-mail: hyhan@mail.hzau.edu.cn*

[b]*State KeyLaboratory of Agricultural Microbiology, College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Wuhan, 430070, P.R. China. Fax: +86 27 87282608; Tel: +86 27 87286974; E-mail: heqigai@yahoo.com;*

‡ Equal contribution by the first two authors.

Experimental Details

Reagents

$CdCl_2 \cdot 2.5H_2O$ (99.0%, Sinopharm), $NaBH_4$ (96%, Sinopharm), Na_2TeO_3 (97%, Sinopharm), 3-mercaptopropionic acid (MPA, 99%, Aldrich), folic acid (FA, Aldrich), N-hydroxysuccinimide (NHS, Aldrich), 1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide Hydrochloride (EDC, Aldrich), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Aldrich) and dimethylsulfoxide (DMSO, Sinopharm) were all commercially available. DMEM was purchased from Sino-American Biotechnology Company. Cells were obtained from Cell Bank of Chinese Academy of Sciences. All chemicals concerned were of analytical grade or the highest purity available and used without further purification. Besides, ultra pure water (Millipore) with a conductivity of 18 MΩ cm was used through out the experiments.

Preparation of CdTe/CdS core/shell QDs

All glasswares were cleaned in a bath of freshly prepared aqua regia ($HCl:HNO_3$, 3:1 by volume) and rinsed thoroughly in Millipore water prior to use. A typical synthesis is described as follows: First, 74 μL MPA and 20 mg trisodium citrate were added to 100 mL $CdCl_2$ solution (0.5 mmol) in succession. After that, the mixture was stirred for 10 min, followed by adding 0.1 M NaOH to tune the pH of the mixed solution to 11.2. Then, 4.4 mg Na_2TeO_3 and 20 mg $NaBH_4$ were added into the mixture under rapid stirring. The molar ratio of Cd^{2+}/MPA/TeO_3^{2-} was fixed at 1:1.7:0.4. Finally, the reaction mixture was slowly heated to 90 °C. Different emission spectra (475 to 810 nm) were obtained by varying the refluxing time. The samples were carefully purified before subsequent measurement and cellular experiments. For centrifugation, QD solution/ethanol was 1:3 in volume at 8,000 rpm.

Conjugation of QD700 (QDs with emission maximum at 700 nm) with FA

QD700 were conjugated to FA following a standard procedure. To 5 mL of 1 mg mL^{-1} QD700 solution, 50 μL of EDC (0.05 M solution in PBS) and an equal amount of NHS (0.05 M solution in PBS) were added and stirred for half an hour. Subsequently, 50 μL of FA (0.05 M solution in DMSO) was added and stirred overnight at room temperature. The unreacted materials were separated out by dialysis against PBS for 3h with 35KD dialysis tubing. The concentration of PBS was 0.01 M.

Characterization

Absorption of QD samples were recorded at room temperature on the Nicolet Evolution 300 UV-Vis spectrometer .The photoluminescence (PL) spectra and lifetime measurements of QDs were performed on an Edinburgh FLS920 spectrometer with an integrating-sphere attachment

under excitation of 370 nm. Measurements of hydrodynamic diameters of the QDs in aqueous solution were acquired on a Malvern Zetasizer Nanoseries using 633 nm laser at 25 °C. TEM and HRTEM analysis of purified QDs were recorded with using a JEM-2010FEF transmission electron microscope operating at an acceleration voltage of 200 kV. XRD patterns were taken on a Rigaku D/MAX-RB diffractometer with Cu Kα radiation (λ=0.15406 nm). XPS were measured by a Thermo VG Multilab 2000 spectrometer equipped with a monochromatic Al K a radiation source at room temperature. The PL QY of the as-prepared QDs was determined through comparison using a Rhodamine G6 standard organic dye.

Cell and animals

Cells that overexpress folate receptor were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% heat-inactivated fetal bovine serum (FBS) and antibiotics (100 mg mL^{-1} streptomycin and 100 U mL^{-1} penicillin) at 37 °C in the humidified atmosphere with 5% CO_2. To establish tumor xenografts, 3×10^6 cells/0.1 mL of HCCLM6 cells were injected subcutaneously into male BALB/C-nu/nu nude mice, 4-5 weeks old, which were maintained in Animal Biosafety Level 3 Laboratory at the Animal Experiment Center of Wuhan University. The animal study protocol was approved by the Animal Welfare Committee of the Center. Two weeks after tumor cell inoculation, subcutaneous tumor mass grew to 0.5 cm in diameter, and the mice were ready for the *in vivo* imaging study detailed below. All animals acclimated to the animal facility for at least 48 h prior to experimentation.

MTT assay

For MTT assay, cells were cultured in 96 well plates and then were incubated up to about 16 h and grown to about 80% confluence before experiments. QD-FA dispersed in DMEM and different

concentrations were added to each well to achieve a final concentration. Cells were then incubated in these medium containing QD-FA at 37 °C and in 5% CO_2 atmosphere for 24 h. After incubation, all cells were washed with PBS to remove excess QD-FA and placed in fresh solutions 200 μL before next experiments. With different incubation time, 20 μL stock MTT (5 mg mL^{-1}) was added to each well, and cells were then incubated for 4 h at 37 °C. The supernatant was abandoned, and 150 μL of DMSO per well was added to dissolve the produced formazan and the plates were shaken for an additional 10 min, and the absorbance of the purple formazan was recorded on Enzyme-linked immunosorbent detector at 490 nm.

Acute toxicity

To assess the acute toxicity of the QD-FA probes *in vivo*, 10 nude mice were randomized into test group and control group (five in each group). After 2 days of adaptation, QD-FA probes were injected into the tail vein of the five mice of test group. QD-FA solution was diluted with saline buffer. The concentration of probes was 1mg mL^{-1} and the dose was 20 mL kg^{-1}. The control mice were injected normal saline under the same condition. The animals were carefully monitored daily for 9 days, and any abnormalities were recorded.

Histological analysis

In histological studies, 10 nude mice were divided into test group and control group (five in each group). After 2 days of adaptation, QD-FA probes were injected into the tail vein of the five mice of experimental group. The concentration was 0.5 mg mL^{-1} and the dose was 20 mL kg^{-1}. The control mice were injected normal saline under the same condition. The major tissue (liver, spleen, lungs, kidneys, heart and brain) were harvested, fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin after injection of the QD-FA for 24 h. The

images were obtained using a spectral microscope (DM 4000M, LECIA Microsystems CMS GmbH).

***In vivo* active tumor imaging**

The tumor bearing nude mouse was anesthetized by intra-abdominal injection of 3% pentobarbital sodium (45 mg kg^{-1}). QD-FA (0.5 mg mL^{-1}) was injected at the dosage of 20 mL kg^{-1} into the tail vein. All the *in vivo* images were taken with a Maestro2 *In Vivo* imaging system (CRi, USA).

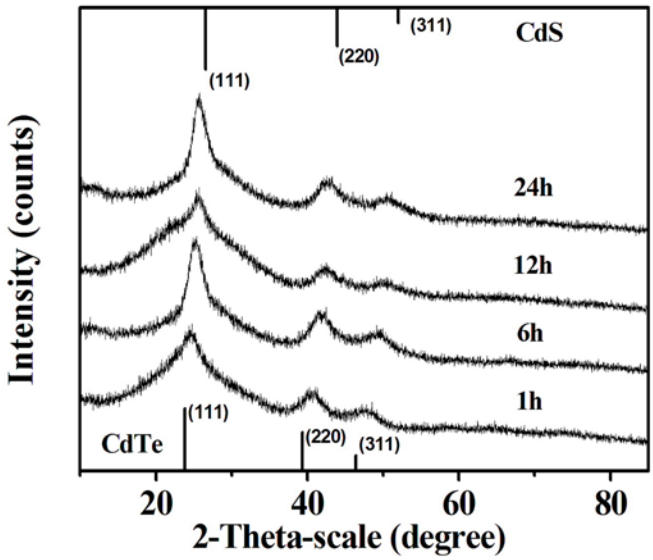

Fig. S1 XRD patterns of the prepared CdTe/CdS QDs reacted for 1, 6, 12 and 24 h.

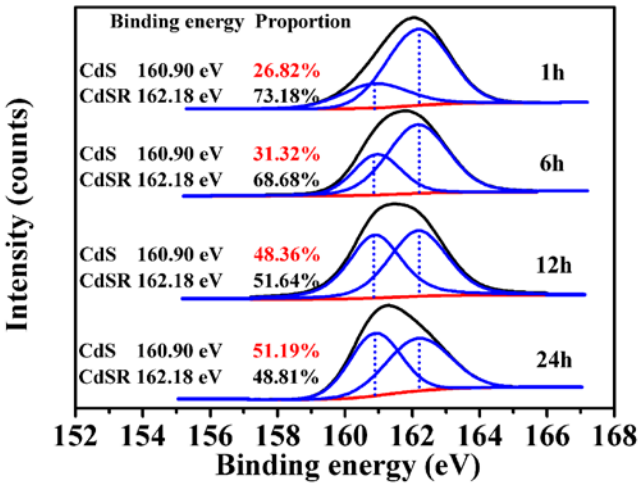

Fig. S2 XPS spectra of S (2p) of the prepared CdTe/CdS QDs reacted for 1, 6, 12 and 24 h.

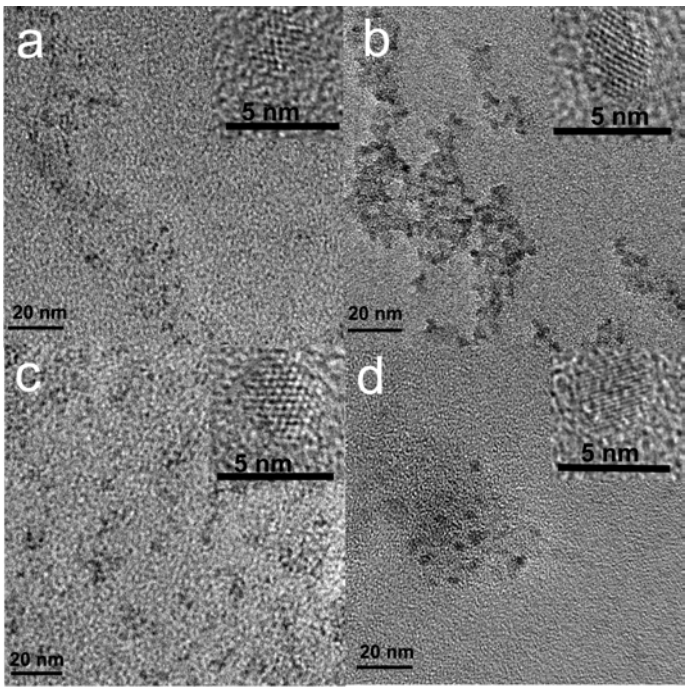

Fig. S3 TEM images and the corresponding HRTEM images (inset) of the prepared CdTe/CdS QDs reacted for (a) 3, (b) 6, (c) 12 and (d) 24 h.

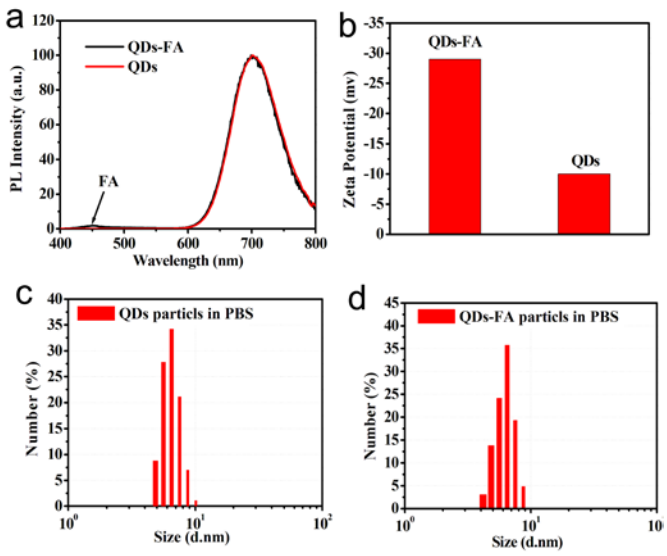

Fig. S4 (a) PL spectra and (b) Zeta potentials of QD700 and QD-FA probes, respectively; dynamic light scattering analysis of (c) QD700 and (d) QD-FA probes in PBS.

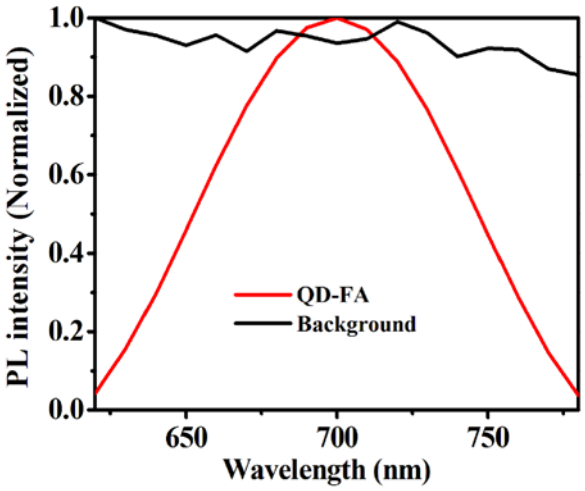

Fig. S5 Spectrum profiles of liver-tumor-bearing nude mice at 4h after injection of the QD-FA probes.