Electronic Supplementary Information

Facile and controlled synthesis of stable water-soluble cupric sulfide quantum dots for significantly inhibiting proliferation of human cancer cells

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Materials and methods

Synthesis of precursor

Firstly, the copper chloride dihydrate (1.550 g, BEIJING HUAGONGCHANG, China) was added into the absolute ethanol (150 mL) and thiaocetamide (TAA) (1.602 g, Sinopharm Chemical Reagent Company) was added into the absolute ethanol (200 mL) under stirring until completely dissolved, respectively. Then two solutions were mixed to form the precursor in the ultrasound for 10 min and the color of solution gradually changed from caramel to milk. The precursor is quite stable in the absolute ethanol under the condition of seal. When it is exposed to the air, it is unstable and its color change gradually from pale yellow to black. The reason could be that it reacts with the water or oxygen in the air.

Synthesis of amorphous CuS QDs

300 ml cold (about 7 ℃) precursor were added into 300 ml cold (about 7 ℃) deionized water under stirring. The suspension reacted at 5 ℃ for 24 h. Then the black precipitates were centrifuged at 20000 rpm and washed with deionized water and absolute ethanol several times and finally dried by vacuum freeze-drying method.

Synthesis of crystalline CuS QDs

300 mL precursor were added into 300 mL deionized water under stirring. Then the suspension was heated at 40 ℃ for 30 min. After cooling down to room temperature, the black precipitates were centrifuged at 20000 rpm and washed with deionized water and absolute ethanol several times and finally dried in vacuum at 40 ℃. As a result, the black hydrosoluble quantum dots were obtained.

Characterization

The identity and the phase of the samples were verified by the X-ray powder diffraction (XRD) on a Bruker D8 Advance X-ray diffractometer with Cu Ka radiation (λ = 1.5406Å). The transmission electron microscopy (TEM), high-resolution transmission electron microscopy (HRTEM) and selected area electron diffraction (SAED) images were received on a microscope (JEOL, JEM-2010). Energy dispersive X-ray spectroscopy (EDS) was performed on a GENESIS system (EDAX Inc.) attached to the JEM-2100 microscope. The photoluminescence (PL) measurements were carried out on a HITACHI FP-6500 spectrophotometer.

Cell culture

Hep G2 human hepatocellular carcinoma cells (ATCC No. HB-8065), Hela human cervical cancer cells and s180 mice glioma cells were cultured in RPMI 1640 medium supplemented with heat-inactivated FBS (10%), Penicillin (100 units/mL), Streptomycin (100 μg/mL), amphotericin B (fungizone, 0.25 μg/mL) and sodium bicarbonate (2 mg/mL) in a humidified incubator at fully humidified atmosphere at 37 ℃, 5% CO_2 and 95% room air. V79 Chinese hamster lung cells (ATCC No. CCL-93) were cultured in DMEM medium supplemented with 10% heat-inactivated FBS, Penicillin (100 units/mL), Streptomycin (100 μg/mL), amphotericin B (fungizone, 0.25 μg/mL) and sodium bicarbonate (3.7 mg/mL) in a humidified incubator at fully humidified atmosphere at 37 ℃, 5% CO_2 and 95% room air.

Treatment of Hela, Hep G2,s180 and V79 Cells with amorphous and crystalline CuS QDs

For Hela and s180 cells, fifty microliters cells with initial cell density of 2.5×10^4 cells/mL were seeded

separately in the wells of sterile 96-well flat bottom culture microplates and incubated with fifty microliters of amorphous and crystalline CuS QDs at final concentrations of 1.5625, 3.125, 6.25, 12.5 25, 50 and 100 μg/mL each for 72 hours in a humidified incubator at fully humidified atmosphere at 37 ℃, 5% CO_2 and 95% room air. For Hep G2 and V79 cells, one hundred microliters of culture media containing cells, Hep G2 or V79, with initial cell density of 2.5×10^4 cells/mL were seeded separately in the wells of sterile 96-well flat bottom culture microplates and acclimated for 48 hours. It was then mixed with one hundred microliters of amorphous and crystalline CuS QDs at concentrations of 0.78125, 3.125, 6.25, 12.5, 25 and 37.5 μg/mL each for 72 hours of incubation in a humidified incubator at fully humidified atmosphere at 37 ℃, 5% CO_2 and 95% room air. The treatment of the cells with culture medium rather than CuS was perpared as the control. The cytotoxic effects of CuS QDs on Hela, HepG2, s180 and V79 cells were determined by MTT colorimetric assay.

MTT colorimetric assay

For cells, after treated with CuS QDs, freshly prepared MTT(3-(4,5-dimethylthiazol-2-yl)-2,5,diphenyl tetrazolium bromide, 20 μL, 5 mg/mL, Sigma-Aldrich, Co., MO, USA) in filtered PBS was added to each well of the control and the CuS-treated cells and incubated at 37 ℃, under 5% CO_2 for 5 hours. After the incubation, the medium was carefully removed by centrifuged for 15 min with 300 g. Then, 150 microliters of DMSO were added to each well to dissolve the dark blue crystals completely. The absorbance of the solution in each well at the wavelength 570 nm was measured by a microplate reader. The extent of cell proliferation was reflected by the average value of absorbance while the cytotoxic effects of amorphous and crystalline CuS QDs were calculated.

Flow cytometric analysis of apoptosis

Hep G2 cells with the cell density of 1×10^6 cells/mL were seeded in culture medium and acclimated for 24 hours. The cells were then incubated with amorphous or crystalline CuS QDs for 48 hours in a humidified incubator at fully humidified atmosphere at 37 ℃, 5% CO_2 and 95% room air. Cells without addition of amorphous or crystalline CuS QDs were prepared as the control. After treatment, Hep G2 cells were trypsinized with trypsin-EDTA solution (0.25%) firstly. All Hep G2 cells were collected and pelleted by centrifugation at 400 g and 10 ℃for 5 minutes. The cell pellets were washed with cold PBS, fixed with ethanol (70%, 3 mL) precooled to -20 ℃, and incubated at -20 ℃ for at least 1 hour. The fixed cells were centrifuged and washed with cold PBS. The cells were stained with propidium iodide (PI, 10 μg/mL in PBS, Sigma-Aldrich, Co., MO, USA) fluorescein isothiocyanate (FITC, 10 μg/mL in PBS, Sigma-Aldrich, Co., MO, USA) at 37 ℃ in the dark for 30 minutes. The stained cells were filtered through 41 μm Nylon net filters and analyzed by a COULTER Epics XL flow cytometry system (Beckman Coulter, Inc., CA, USA) in sextuplicate and the percentages of quarant were recorded.

Measurement of apoptosis using confoeal laser scanning microscopy

Hep G2 cells were incubated together with amorphous or crystalline CuS QDs in culture medium in a 96-well round bottom microplate for 48 hours (sextuplicate) in a humidified incubator at fully humidified atmosphere at 37 ℃, 5% CO_2 and 95% room air. After the treatment, cell mixtures were pelleted by centrifugation (200 g, 10 min) and the supernatant were carefully removed except for approx 25–30 μL. Ten microlitres of this mixture were placed on clean microscope slides and were covered with a coverslips. The slides were examined with different objectives (40× to 60×) using the epiilumination and a filter combination at 340-380 nm.

Results

CdS, ZnS QDs were also obtained using this facile hydrolysis synthesis strategy. From Fig. S1, we can know the QDs sizes are about 5 nm.

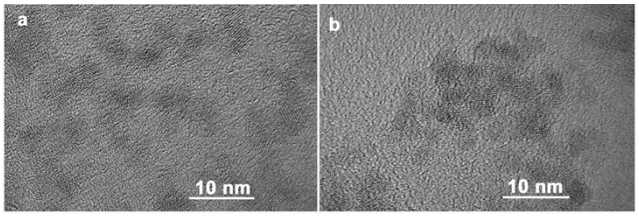

Fig. S1 TEM of (a) CdS QDs,(b) ZnS QDs.

The precursor was characterized by X-ray diffraction (XRD) as shown in Fig. S2. The XRD patterns of the precursor present a few strong diffraction peaks. Through analyzing XRD patterns, the precursor is composed of $Cu_3(TAA)_3Cl_3$.

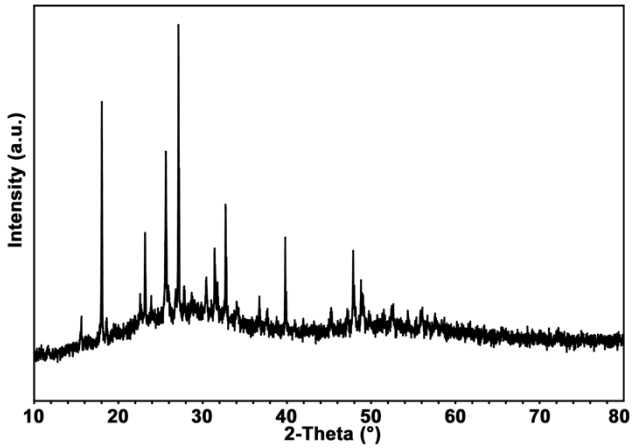

Fig. S2 XRD patterns of $Cu_3(TAA)_3Cl_3$.

The ingredients of the amorphous and crystalline CuS QDs were further confirmed by the energy dispersive spectrocopy (EDS) measurement. From the results shown in Fig. S3, the atom ratio of Cu to S is about 1:1 both for the amorphous QDs and crystalline QDs. This result indicates that the obtained samples are cupric sulfide (CuS) without impurities.

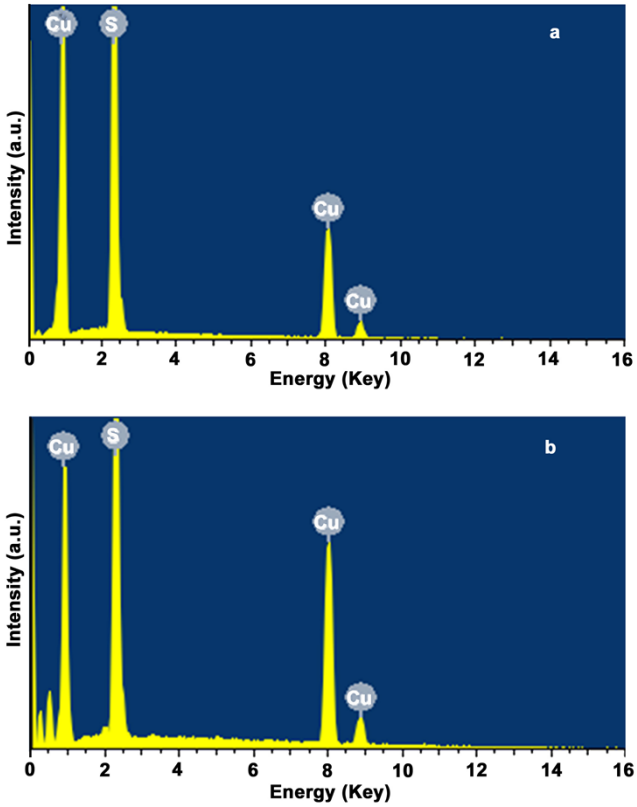

Fig. S3 EDS images of (a) amorphous CuS QDs, (b) crystalline CuS QDs.

The zeta potentials of amorphous and crystalline CuS QDs in PBS, FBS, DMEM are measured in (Table S1, ESI†). From the result, we can know, the zeta potentials of amorphous QDs are higher.

Table 1 The zeta potentials of CuS QDs in different physiological solutions.

	PBS	FBS	DMEM
Amorphous	-27.0 mV	-21.3 mV	-24.7 mV
Crystals	-23.2 mV	-19.6 mV	-21.8 mV

In order to investigate the photo-stability of these QDs, we measured the PL spectra at different time (Fig. S4). The QDs were dispersed in distilled water before measuring the PL spectra. From the Fig. S4, we can know the intensity of these QDs rarely weaken even after 300 min. The result shows the photo-stability of these QDs is relatively good.

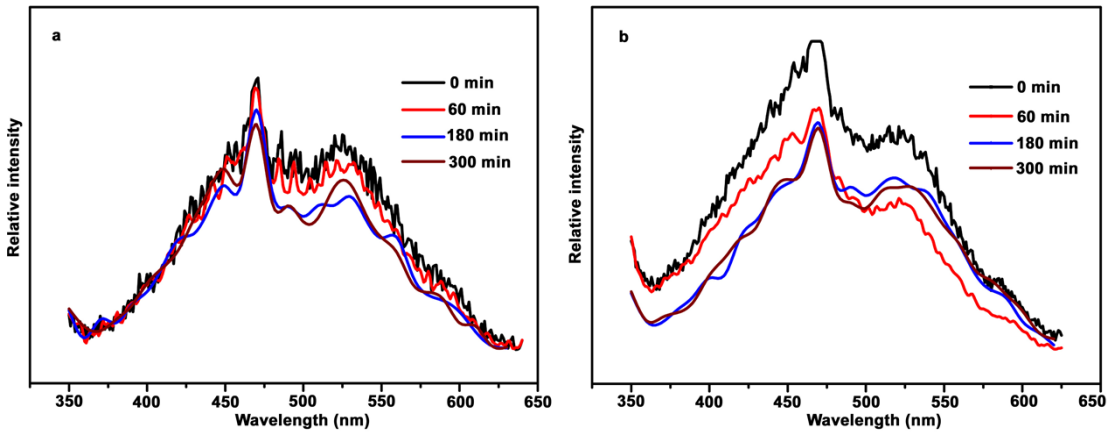

Fig. S4 PL spectra of CuS QDs, (a) amorphous, (b) crystals at different time.