Electronic Supplementary Materials

Silica-based nanoarchitecture for an optimal combination of photothermal and chemodynamic therapy functions of $Cu_{2-x}S$ cores with red emitting carbon dots

Alexey Stepanov (✉)[1], Svetlana Fedorenko[1], Kirill Kholin[2], Irek Nizameev[3], Alexey Dovzhenko[4], Rustem Zairov[1,4], Tatiana Gerasimova[1], Alexandra Voloshina[1], Anna Lyubina[1], Guzel Sibgatullina[5], Dmitry Samigullin[3,5], Asiya Mustafina[1]

1 Arbuzov Institute of Organic and Physical Chemistry, FRC Kazan Scientific Center of RAS, Kazan 420088, Russia

2 Department of Physics, Kazan National Research Technological University, Kazan 420015, Russia

3 Department of Nanotechnology in Electronics, Kazan National Research Technical University named after A.N. Tupolev-KAI, Kazan 420111, Russia

4 Kazan (Volga region) Federal University, Kazan 420008, Russia

5 Kazan Institute of Biochemistry and Biophysics, FRC Kazan Scientific Center of RAS, Kazan 420111, Russia

E-mail: aleksestepanov@yandex.ru

1. Experimental

Safety and hazards note

The synthesis of copper sulfide nanoparticles requires the use of Schlenk line to evacuate the air from the reaction flask and then backfill it with Ar. Besides that, this synthesis is carried out at about 190ºC. For these reasons, personnel must be qualified to do this kind of work, must wear protective goggles and follow other precautions during the synthesis in compliance with safety regulations at a specific workplace. All other experiments must be performed taking into consideration safety rules and regulations which are common practice in chemical laboratories.

1.1. Materials

Commercial chemicals tetraethyl orthosilicate (TEOS) 98%, ammonium hydroxide (28-30%), n-hexanol 98%, cyclohexane 99%, hexane (99%), acetone (99%), oleic acid (97%), oleylamine, sulfur (99.9%), copper (I) chloride (99%), 1-octadecene (90%), 3-aminopropyltriethoxysilane (APTES, 99%), tris(2,2'-bypyridyl)ruthenium(II) chloride, fluorescamine, citric acid, urea, dimethylformamide, cetyltrimethylammonium bromide

CTAB) were purchased from Acros Organics. Sodium oleate (95%) were bought from Sigma-Aldrich. Mito-tracker Green FM was purchased from Thermo Fisher Scientific.

Ethanol was purified by distillation under atmospheric pressure. Dimethylformamide was purified under reduced pressure. All other chemicals were used as received without further purification.

2. Preparation methods

2.1. Synthesis of $Cu_{2-x}S$ nanoparticles

The plasmonic $Cu_{2-x}S$ nanoparticles were synthesized by means of the published method [23]. Briefly, the hot solution of CuCl in a mixture of oleic acid and oleylamine was injected into hot solution of S in octadecene under vigorous stirring with subsequent refluxing for several minutes. At some point in time the reaction mixture turns black indicating the formation of $Cu_{2-x}S$ nanoparticles. For more detailed description of the synthetic procedure see our previous article [23].

2.2. Synthesis of carbon dots

Synthesis of carbon dots was performed according to literature method [25]. Briefly, 1 g of citric acid and 2 g of urea were dissolved in 10 mL $HC(O)N(CH_3)_2$ using ultrasound water bath. The obtained solution was kept refluxing at 160ºC for 6 hours. After being cooled to room temperature the solution was mixed with 10 mL of NaOH aqueous solution (C = 0.1 $g \cdot mL^{-1}$) and centrifuged. The obtained precipitate was washed several times with distilled water and dried at about 60ºC.

2.3. Solubilization of $Cu_{2-x}S$ nanoparticles with CTAB

0.0602 g of the synthesized $Cu_{2-x}S$ nanoparticles were dispersed in 2.41 mL of $CHCl_3$ for 30 min. Then, 12.04 mL of CTAB aqueous solution (5 $g \cdot L^{-1}$) was added to the previously prepared dispersion. The obtained mixture was ultrasonicated for 40 min. Next, $CHCl_3$ was removed using rotary evaporator which resulted in the formation of CTAB-stabilized aqueous dispersion of $Cu_{2-x}S$ nanoparticles.

2.4. Synthesis of $Cu_{2-x}S@SiO_2NH_2$ nanoparticles

5 mL of H_2O, 1.5 mL of C_2H_5OH and 0.116 mL of 1.3 M NaOH water solution were added to 10 mL of CTAB-stabilized aqueous dispersion of $Cu_{2-x}S$ nanoparticles (0.5 $g \cdot L^{-1}$). The mixture was heated up to 60ºC, afterwards, 0.1 mL of TEOS was added, and the mixture was kept at 60ºC for 2 hours. After that, the obtained nanoparticles were washed with 16 mL of C_2H_5OH by means of 6 centrifugation (14000 rpm, 15 min, 4ºC) and re-dispersion steps. Next, C_2H_5OH (16 mL) was

added to the obtained nanoparticles, and the mixture was dispersed using ultrasound water bath for 10 min. Then, APTES (0.03 mL) was added to 5 mL of the prepared $Cu_{2-x}S@SiO_2$ dispersion, and the obtained mixture was left under stirring for 18 hours (700 rpm). The synthesized colloids were washed 3 times with 16 mL of C_2H_5OH and 2 times with 16 mL of H_2O. The concentration of the synthesized aqueous colloid was found to be at 5 $g \cdot L^{-1}$. The concentration of NH_2 groups was determined by the fluorescence procedure with use of fluorescamine [26] and amounted to be about $1{,}86 \cdot 10^{-3}$M for 1 $g \cdot L^{-1}$ dispersion.

2.5. Synthesis of CDs-modified $Cu_{2-x}S@SiO_2NH_2$ nanoparticles

First of all, 5 mL of $Cu_{2-x}S@SiO_2NH_2$ aqueous dispersion (0.5 $g \cdot L^{-1}$, acidified with HCl to pH 4.0) and aqueous dispersion of carbon dots (0.5 $g \cdot L^{-1}$) have been prepared, mixed together and ultrasonicated for 15 minutes. Then, the mixture was vigorously shaken (~ 700 rpm) using a shaker set-up for 4 hours. The resulting colloid has been centrifuged (15000 rpm, 20 min, 4ºC), supernatant solution discarded and the remaining precipitate has been washed with 15 mL of doubly-distilled water by 3 centrifugation-redispersion steps. After the last centrifugation step the solid precipitate has been dispersed in 10 mL of doubly-distilled water for 30 minutes resulting in a desired aqueous colloid. The concentration of obtained colloid was determined to be about 0.243 $g \cdot L^{-1}$.

3. Characterization techniques

3.1. The investigation of photothermal effect of $Cu_{2-x}S@SiO_2NH_2$CDs nanoparticles

The photothermal effect of $Cu_{2-x}S@SiO_2NH_2$CDs nanoparticles has been studied with the help of determining the temperature of 1.5 mL of aqueous colloid with concentrations 0.243, 0.200, 0.120, 0.06, 0.03 $g \cdot L^{-1}$ in a glassy cuvette under irradiation with 1064 nm light emitting diode under vigorous stirring. The light power controller is DPSSL DRIVER FA device, whereas the source of 1064 nm NIR light is infrared laser module LSM-LMi-1064D-5000 equipped with 220 V power supply and optical fiber with the length of 1 m. Power density is equal to 1.5 W/cm^2.

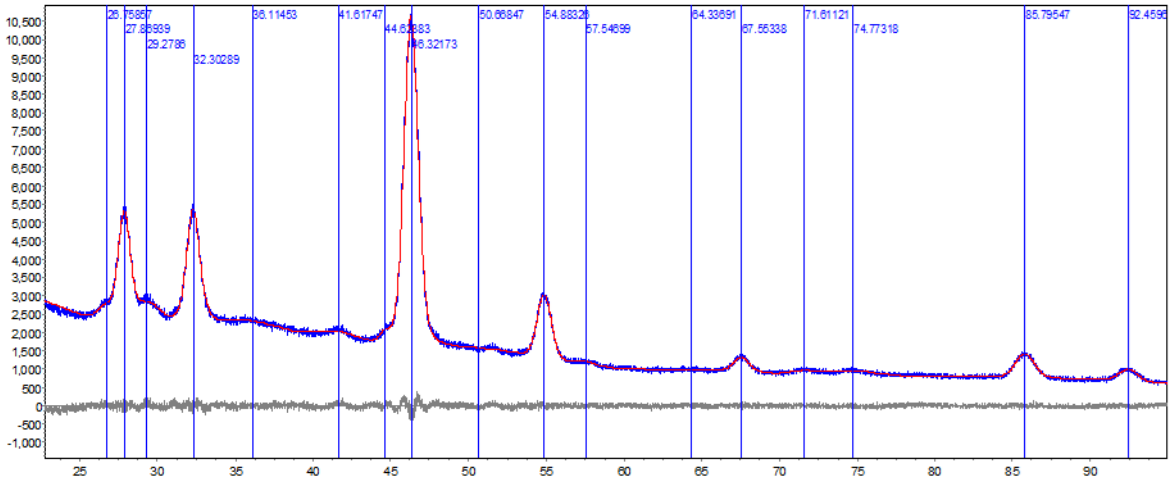
The photothermal effect of each of the above-mentioned nanoparticles' concentrations was estimated by three 20 minutes consecutive cycles, namely, heating, cooling, heating. So, one measurement took 60 minutes. The temperature of colloids under irradiation was recorded every minute with use of contact thermometer (CENTER 303, Type K J, Thermometer, Taiwan). The sensitivity of this thermometer is 0.1ºC. The same conditions were applied when measuring the temperature rise (drop) of doubly-distilled water as a control measurement.

3.2. XRD measurements

X-ray diffraction measurements have been conducted employing Bruker D8 Advance diffractometer having Vario attachment and Vantec linear PSD, using Cu radiation (40 kV, 40

mA) monochromated by the curved Johansson monochromator (λ Cu $K_{\alpha 1}$ 1.5406 Å). Room-temperature data were collected in the reflection mode with a flat-plate sample. The sample was loaded on a standard zero diffraction silicon plate, which was kept spinning (15 rpm) during the data collection. Patterns have been recorded in the 2Θ range between 3o and 93o, in 0.008o steps, with a step time of 0.1–4.0s. A few diffraction patterns in various experimental modes have been obtained and summed for the sample. Processing of the data collected has been made with the help of EVA and TOPAS software packages. The PDF-2 powder X-ray diffraction database (ICDD PDF-2, Release 2005-2009) was used to identify the crystalline phase.

The crystallites sizes calculations have been carried out by means of the TOPAS software package in several ways: the values calculated from the half-width of the reflections (LVol-FWHM) and the integrated reflection intensity (LVol-IB) are the volume-weighted values of the crystallite sizes, and the CrySizeG parameter is the size of the crystallites in the direction perpendicular to the analyzed planes, with the Gauss type of peak broadening. The minimization of the discrepancy between the experimental and calculated data in the refinement process was performed by the Rietveld method over the entire array of experimental data. The correctness of the comparison of the model (theoretical) and experimental curves was ensured by minimizing the convergence parameters Rwp and Rexp. The difference curve serves as an additional visual criterion for the convergence of the results.



Experimental XRD pattern of the sample (blue curve) and theoretical curve (red curve). Vertical lines show the positions of the interference peaks corresponding to the crystalline phase. The gray curve is the residual difference curve.

3.3. FTIR measurements

The powder samples of about 1.5 mg mass were mixed with approximately 150 mg of solid KBr and pressed into pellets. The corresponding FTIR spectra were recorded in the transmittance mode at the Bruker Tensor 37 FTIR spectrometer in the 400 – 4000 cm^{-1} wavenumber range, optical resolution 4 cm^{-1}.

3.4. NIR measurements

The near infrared (NIR) spectra of $Cu_{2-x}S$ samples in cyclohexane (C = 0.5 $g \cdot L^{-1}$) and $Cu_{2-x}S@SiO_2NH_2$ dispersions in D_2O (C=1 $g \cdot L^{-1}$) have been recorded in the transmittance mode at the Bruker Tensor 37 FTIR spectrometer in the 4000 – 15000 cm^{-1} (2500 – 666 nm) range, optical resolution 4 cm^{-1}.

3.5. pH-metry

pHs of the solutions were determined by means of Microprocessor pH meter «pH 212» (Hanna Instruments, Germany). Before each measurement, the pH-meter was calibrated with standard aqueous buffer solutions (pHs 7.01 and 4.01) (25°C).

3.6. UV-vis measurements

The UV–vis measurements were carried out using Lambda 35 spectrophotometer (Perkin-Elmer, USA) with 10 mm cuvettes at 25°C.

3.7. ICP-OES analysis

Cu, Ru and Si were identified in the colloids by means of simultaneous inductively coupled plasma optical emission spectrometry (ICP-OES), model iCAP 6300 DUO by Varian Thermo Scientific Company equipped with a CID detector. This spectrometer can simultaneously measure the peak heights within the 166 to 867 nm range. The optical resolution is less than 0.007 nm to 200 nm. The working frequency is 27.12 MHz. Together, the radial and axial view configurations enable optimal peak height measurements with suppressed spectral noises. Concentrations of Cu, Ru and Si were found by the spectral lines: 327.396, 267.876 and 251.611 nm, respectively.

3.8. Steady-state emission

The steady-state emission spectra were recorded on a fluorescence spectrophotometer Hitachi F-7100 (Japan) with stigmatic concave diffraction grating (www.hitachi-hightech.com/global/product_detail/?pn=ana-f7100). All samples were ultrasonicated for 15 min before measurements. The fluorescence procedure with use of fluorescamine was employed in order to determine the number of amino groups onto the surface of silica nanoparticles.

3.9. Dynamic light scattering measurements

Dynamic light scattering (DLS) measurements were performed by means of the Malvern Mastersize 2000 particle analyzer. A He–Ne laser operating at 633 nm wavelength and emitting vertically polarized light was used as a light source. The measured autocorrelation functions were analyzed by Malvern DTS software and the second-order cumulant expansion methods.

The effective hydrodynamic radius (R_H) was calculated by the Einstein–Stokes relation from the first cumulant: $D = k_BT/6\pi\eta R_H$, where D is the diffusion coefficient, k_B is the Boltzmann constant, T is the absolute temperature, and η is the viscosity. The diffusion coefficient was measured at least three times for each sample. The average error in these experiments is approximately 4%. The samples for DLS study were prepared from deionized water, sonicated for 30 minutes and equilibrated at 25.0 ± 0.1 °C before DLS and zeta-potential measurements.

3.10. ESR experiments

The ESR measurements were performed with use of ELEXSYS E500 ESR spectrometer of the X-range (Bruker, Billerica, Massachusetts, USA). ESR spectra were simulated by means of the WinSim programme (developed by NIEHS, version 0.96). The samples were aqueous (used deionized water) dispersions of 0.1 M DMPO in the presence of analyzed nanoparticles. Temperature in the ESR spectrometer cavity was set using the ER 4131VT Variable Temperature System (Bruker, Billerica, Massachusetts, USA). The temperature control lasted 4 min. The EPR spectra are recorded in 10 min after mixing the investigated system with DMPO, when the system equilibrates and there are no changes in the parameters of the spectra. The countdown in all experiments begins from the moment when the DMPO solution was added to the investigated system.

4. Morphological analyses

4.1. TEM measurements

The sample of $Cu_{2-x}S$ nanoparticles for TEM investigations was prepared as described hereinafter. A $Cu_{2-x}S$ powder sample of about 1 mg mass was dispersed in 6.5 mL of hexane by means of ultrasound. After that, 15 µl of the dispersion was placed on 200 mesh copper grids with continuous formvar support film and hexane was evaporated at room temperature. The TEM images were obtained with use of Hitachi HT7700 (Japan) TEM set-up at an accelerating voltage of 100 kV.

4.2. HRTEM measurements

Firstly, 3 µl of the initial dispersion was 100-fold diluted with ethanol. Then, the obtained dispersion was placed onto 3 mm copper grid with formvar/carbon support film and dried at room temperature during 2 hours. Afterwards, the microscopic measurements were carried out in transmission mode by means of transmission electron microscope Hitachi HT 7700 Exalens at acceleration voltage of 100 kV.

4.3. SEM measurements

The SEM analysis was conducted using auto-emission scanning electron microscope Merlin (Carl Zeiss). This microscope is equipped with an AZtec X-Max energy dispersion spectrometer

(Oxford Instruments). The resolution of the spectrometer is 127 eV. The surface morphology was imaged at an accelerating voltage of 5 keV to improve the image sharpness. In order to obtain high-resolution microphotographs, the investigated nanoparticles were pre-sprayed with an Au/Pd alloy in the 80/20 ratio. The samples, fixed on the holder, were placed in an electron microscope chamber. The probing was carried out from the selected sites.

5. Biological investigations

5.1. Cytotoxicity assays

For cytotoxicity determination, tumour cell cultures of M-HeLa clone 11 cells (epithelioid carcinoma of the cervix, subline HeLa., clone M-HeLa) from the collection of the Institute of Cytology of the Russian Academy of Sciences (St. Petersburg, Russia) and human liver cells (Chang liver) from the collection and the Research Institute of Virology of the Russian Academy of Medical Sciences (Moscow) were used; a line of normal human liver cells (Chang liver) from the collection of the Research Institute of Virology of the Russian Academy of Medical Sciences (Moscow, Russia).

Cytotoxic activity was assessed by reducing the total activity of mitochondrial dehydrogenases of Chang liver cell line in a colorimetric microtetrazole WST test. The method is based on the ability of NADP-H-dependent cellular oxidoreductases to reduce tetrazolium dye (WST-8) - (2-(2- methoxy-4-nitropentyl)-3-(4-nitropentyl)-5-(2,4-disulfophenyl)-2H-tetrazolium) to formazane which is of yellow colour. The cytotoxicity of the tested samples was estimated in accordance with the standard protocol [Cell Counting Kit-8 Cell Proliferation Assay and Cytotoxicity Assay Technical Manual file:///C:/Users/22B5~1/AppData/Local/Temp/Dojindo-Cell-Proliferation-Protocol.pdf]. Calculation of IC50, the concentration of the tested compound that causes cell growth suppression by 50%, was made utilizing the program MLA - "Quest Graph IC50 Calculator". AAT Bioquest, Inc, 15 December, 2022, www.aatbio.com/tools/ic50-calculator.

5.2. Flow cytometry assay

Cell Culture. M-HeLa cells in the amount of 1×10^5 cells/well in a final volume of 2 ml were sown in 6-well plates (Nunc). After 24-hour incubation nanoparticles at a concentration of 0.03 $g \cdot L^{-1}$ were added to the wells and incubated for 24 hours in a CO_2 incubator.

Cellular uptake study of nanoparticles.

Cellular uptake of $Cu_{2-x}S@SiO_2NH_2$CDs nanoparticles were analyzed by flow cytometry (Guava easy Cyte 8HT, USA). Flow cytometry was used to study the uptake of the test object by the cancer cells. Untreated cells M-HeLa were used as a negative control. The test objects were analyzed at a concentration of 0.03 $g \cdot L^{-1}$ with a cell viability of 72-95%.

5.3. Cell Apoptosis Analysis

M-HeLa cells at 1×10^6 cells/well in a final volume of 2 ml were plated in 6-well plates and

incubated for 24 h until a monolayer was formed. Then, $Cu_{2-x}S@SiO_2NH_2$CDs were added to the wells at concentrations of 0.013 $g \cdot L^{-1}$, 0.025 $g \cdot L^{-1}$ and 0.05 $g \cdot L^{-1}$ and kept for 24 h in a CO_2 incubator. Cells were then harvested at 2000 rpm for 5 min, washed twice with ice-cold PBS, followed by resuspension in 100 µl of binding buffer. Next, control and test samples were incubated with 0.35 µl of Annexin V-Alexa Fluor 647 and 0.1 µl of propidium iodide for 40 min at room temperature in the dark. Finally, cells were analyzed by flow cytometry (Guava easy Cyte, MERCK, USA). The experiments were repeated three times.

5.4. Confocal laser microscopy

For imaging, M-HeLa cells were incubated for 24 hours with $Cu_2S@SiO_2NH_2$CDs at a final concentration $3 \cdot 10^{-7}$ $g \cdot L^{-1}$. Then cells were washed twice with DPBS and incubated for 20 minutes with MitoTracker Green (50 $ng \cdot mL^{-1}$, Invitrogen, USA) or DiD (1:1000, Invitrogen, USA). For microscopic observation and image acquisition Leica TCS SP5 confocal microscope (Leica, Germany) equipped with a set of lasers with a wavelength range from ultra-violet to red was used. Mitotracker Green FM was excited at 488 nm and the fluorescence emission was collected from 500 to 540 nm; DiD was excited at 633 nm and the fluorescence emission was collected from 630 to 670 nm. Signal from samples was excited at 461 nm and the fluorescence emission was collected from 600 to 630 nm.

To determine the effectiveness of internalization, the ratio between the number of cells containing nanoparticles and the total number of cells was calculated. The calculations were performed manually using fluorescent images. At least 20 representative observation fields were chosen in each experiment. At least 50 cells were analyzed for each time point.

Co-localization was analyzed using Las AF software (Leica Biosystems, Germany). The Pearson's correlation coefficient was calculated for each captured field of vision (n = 50), at least 50 cells were taken into account for each field. Data are expressed as means ± SE. The significance of differences of data was analyzed with Student's test. Differences were regarded to be statistically significant at $P < 0.05$.

5.5. Statistical analysis

The Pearson's correlation coefficient was calculated for each captured field of vision (n = 50), at least 50 cells were taken into account for each field. Data are expressed as means ± SE. The significance of differences of data was analyzed with Student's test. Differences were regarded to be statistically significant at $P < 0.05$.

The IC50 values were calculated using the online calculator MLA - Quest Graph™ IC50 Calculator AAT Bioquest, Inc, February 14, 2023. Statistical analysis was performed using the Mann-Whitney test ($p < 0.05$). Tabular and graphical data contains averages and standard deviation.

6. Qualitative tests for copper ions release

We have carried out a qualitative test to determine whether copper ions are released from $Cu_{2-x}S$@SiO_2NH_2-CDs into aqueous solution by means of the well-known reactions. Firstly, the $Cu_{2-x}S$@SiO_2NH_2-CDs aqueous suspension (0.243 g/L) was ultrasonicated under air for 1 hour and then vigorously shaken for 8 hours also under air to ensure the oxidation of the Cu^+ ions, which are supposed to release from nanoparticles. Next, the nanoparticles have been centrifuged (12500 rpm, 20 min) and transparent uncolored supernatant solution qualitatively analyzed for Cu^{2+} presence by adding excess amount of NH_3 aqueous solution. After that the tested solution didn't change its colour to blue which indicates negligible, if any at all, release of copper from nanoparticles. This result was also confirmed by the so-called microcrystalloscopic reaction of Cu^{2+} ions. In particular, the analyzed solution was slightly acidified with CH_3COOH and then a few drops of the $(NH_4)_2[Hg(SCN)_4]$ water solution was added. No signs of yellow-green $Cu[Hg(SCN)_4]$ precipitate were observed. These experimental results clearly indicate very negligible release of copper ions form our nanoparticles.

Table S1. DLS data for $Cu_{2-x}S$@CTAB aqueous colloids[a] at different concentrations, as well as for the aqueous dispersions of the CDs at 0.005 $g \cdot L^{-1}$.

Colloid	C, $g \cdot L^{-1}$	D^I/nm[b]	D^N/nm[c]	ξ-potential/ mV	PDI	pH
$Cu_{2-x}S$@CTAB	0.2	193±15 (60.4%) 23±3	16±2	+ 42±2	0.573	5.91
$Cu_{2-x}S$@CTAB	3.72	385±20 (75%) 21±3 (21.5%) 4745 (3.5%)	15±2	+ 130±10	1.0	
CDs (H_2O solution)	0.005	300.2±68 (79.8%) 35.8±5 (8.0%) 4990 (12.2%)	23.8±3	-29.9 ± 7.63	0.479 ± 0.188	6.04

[a]No buffers or electrolytes were added into dispersions for size and ξ-potential measurements

[b]By intensity

[c]By number

Table S2. ICP-OES data.

Sample name	Elements content/mg/L, ±10%		
	Si (251.611 nm)	Cu (324.754 nm)	Ru (267.876 nm)
$Cu_{(2-x)}S$ $MesoSiO_2$-NH_2	8.23	30.0	-
$Cu_{(2-x)}S$ $MesoSiO_2$-OH	3.97	14.1	-
$Cu_{(2-x)}S$ Rudip$MesoSiO_2$-NH_2	6.00	9.10	0.021

Table S3. Cell viability values measured for M-HeLa and Chang Liver cell lines incubated with $Cu_{2-x}S@SiO_2$-NH_2 and $Cu_{2-x}S@SiO_2$-NH_2-CDs at various concentrations. The IC_{50} values were averaged from the data obtained by two independent measurements designated as 1 and 2.

M-HeLa Compound	Concentration/ $g{\cdot}L^{-1}$	Viability/%	$IC_{50}/g{\cdot}L^{-1}$	$(IC_{50})_{av}/g{\cdot}L^{-1}$
1 $Cu_{2-x}S@SiO_2$-NH_2	0.1215	29.87249545	0.0295	0.03± 0.002
	0.06075	38.97996357		
	0.030375	52.64116576		
	0.0151875	53.91621129		
	0.00759375	73.95264117		
	0.0037968	75.73770492		
2 $Cu_{2-x}S@SiO_2$-NH_2	0.1215	26.77595628	0.0317	
	0.06075	42.25865209		
	0.030375	50.09107468		
	0.0151875	58.83424408		
	0.00759375	76.50273224		
	0.0037968	79.74499089		
Chang Liver Compound	Concentration/ $g{\cdot}L^{-1}$	Viability %	$IC_{50}/g{\cdot}L^{-1}$	$(IC_{50})_{av}/g{\cdot}L^{-1}$
1 $Cu_{2-x}S@SiO_2$-NH_2	0.1215	52.10526316	0.157	0.13±0.04
	0.06075	60.78947368		
	0.030375	67.63157895		
	0.0151875	64.73684211		
	0.00759375	77.10526316		
	0.0037968	74.47368421		
2 $Cu_{2-x}S@SiO_2$-NH_2	0.1215	43.68421053	0.0942	
	0.06075	59.47368421		
	0.030375	66.31578947		
	0.0151875	61.31578947		
	0.00759375	81.31578947		
	0.0037968	86.05263158		
HeLa Compound	Concentration/ $g{\cdot}L^{-1}$	Viability/%	$IC_{50}/g{\cdot}L^{-1}$	$(IC_{50})_{av}/g{\cdot}L^{-1}$
1 $Cu_{2-x}S@SiO_2$-NH_2-CDs	0.1215	43.2	0.1	0.09±0.01
	0.06075	100		
	0.030375	100		
	0.0151875	100		
	0.00759375	100		
	0.0037968	100		
2 $Cu_{2-x}S@SiO_2$-NH_2-CDs	0.1215	42.83054	0.08	
	0.06075	100		
	0.030375	100		
	0.0151875	100		
	0.00759375	100		
	0.0037968	100		
Chang Liver Compound	Concentration/ $g{\cdot}L^{-1}$	Viability/%	$IC_{50}/g{\cdot}L^{-1}$	$(IC_{50})_{av}/g{\cdot}L^{-1}$
1 $Cu_{2-x}S@SiO_2$-NH_2-	0.1215	54.8	0.2	0.1645±0.05
	0.06075	55.2		

CDs	0.030375	75.5		
	0.0151875	76.2		
	0.00759375	85.9		
	0.0037968	90.5		
2 $Cu_{2-x}S@SiO_2$-NH_2-CDs	0.1215	56.6	0.13	
	0.06075	59.6		
	0.030375	75.8		
	0.0151875	81.6		
	0.00759375	88.0		
	0.0037968	90.5		

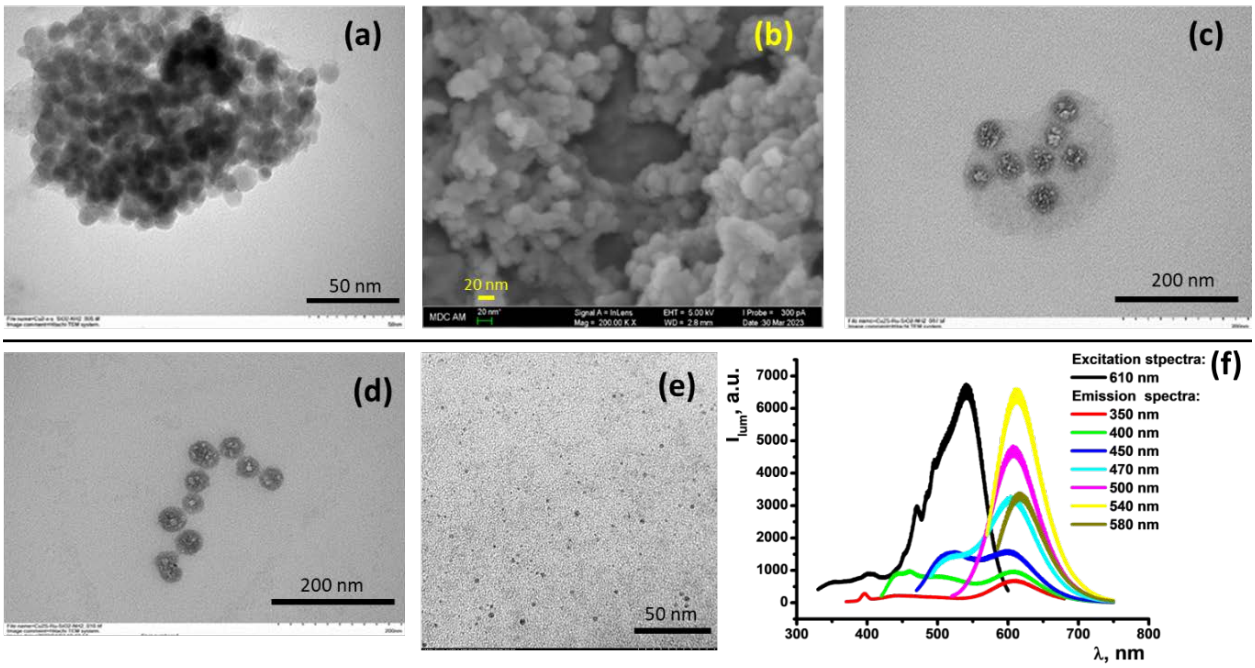


Fig. S1. (a) HRTEM image of $Cu_2S@SiO_2$-NH_2 nanoparticles, (b) SEM image of $Cu_2S@SiO2$-NH_2 nanoparticles, (c) and (d) HRTEM images of $Cu_2SRu@SiO_2$-NH_2 nanoparticles, TEM image (e) and excitation (black, λ_{em} = 610 nm) and emission spectra of the CDs at different excitation wavelengths (f).

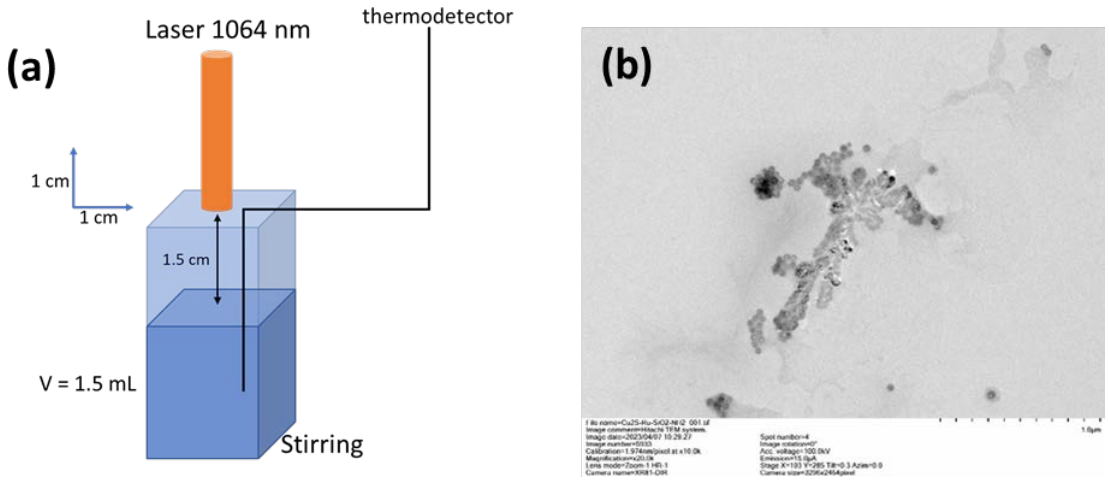


Fig. S2. (a) Schematic representation of photothermal conversion measurements of $Cu_2S@SiO_2$-NH_2-CDs aqueous colloids and (b) TEM image of $Cu_{2-x}S@SiO_2$-NH_2-CDs (0.12 $g \cdot L^{-1}$) after the heating-cooling-heating cycle.

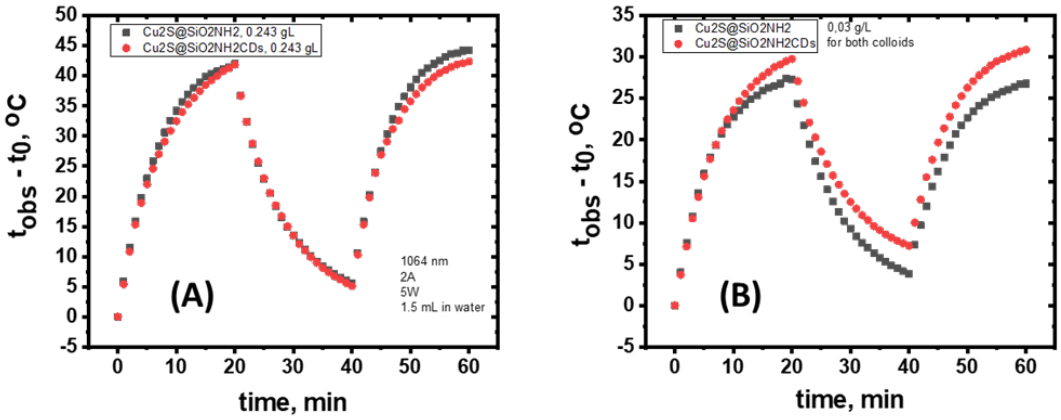


Fig. S3. Panel (A): the photothermal heating curves for $Cu_2S@SiO_2$-NH_2 (black curve) and $Cu_2S@SiO_2$-NH_2-CDs (red curve) nanoparticles in water at the concentration of 0.243 g/L. Panel (B): the photothermal heating curves for $Cu_2S@SiO_2$-NH_2 (black curve) and $Cu_2S@SiO_2$-NH_2-CDs (red curve) nanoparticles in water at the concentration of 0.03 $g \cdot L^{-1}$. λ = 1064 nm, power 1.5 W/cm^2.

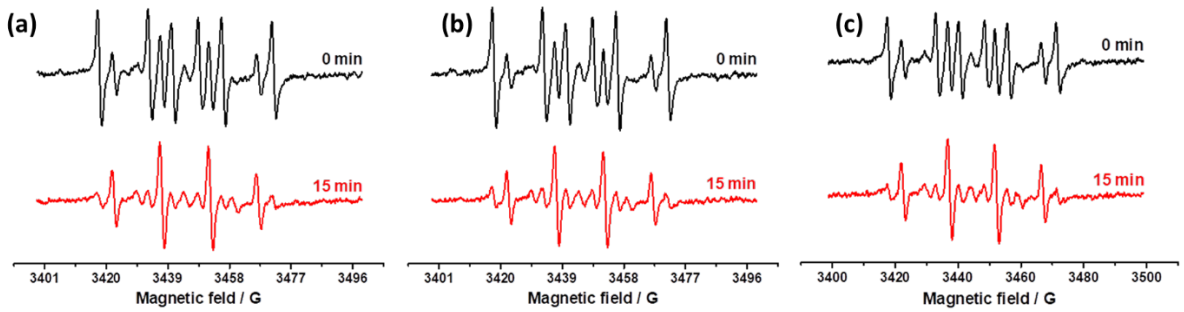


Fig. S4. The (a), (b) and (b) panels represent the EPR spectra of $Cu_2S@SiO_2$-NH_2-CDs nanoparticles in aqueous colloids with concentrations of H_2O_2 being $4.8 \cdot 10^{-5}$, $8.03 \cdot 10^{-5}$, $1.17 \cdot 10^{-4}$ M, respectively.

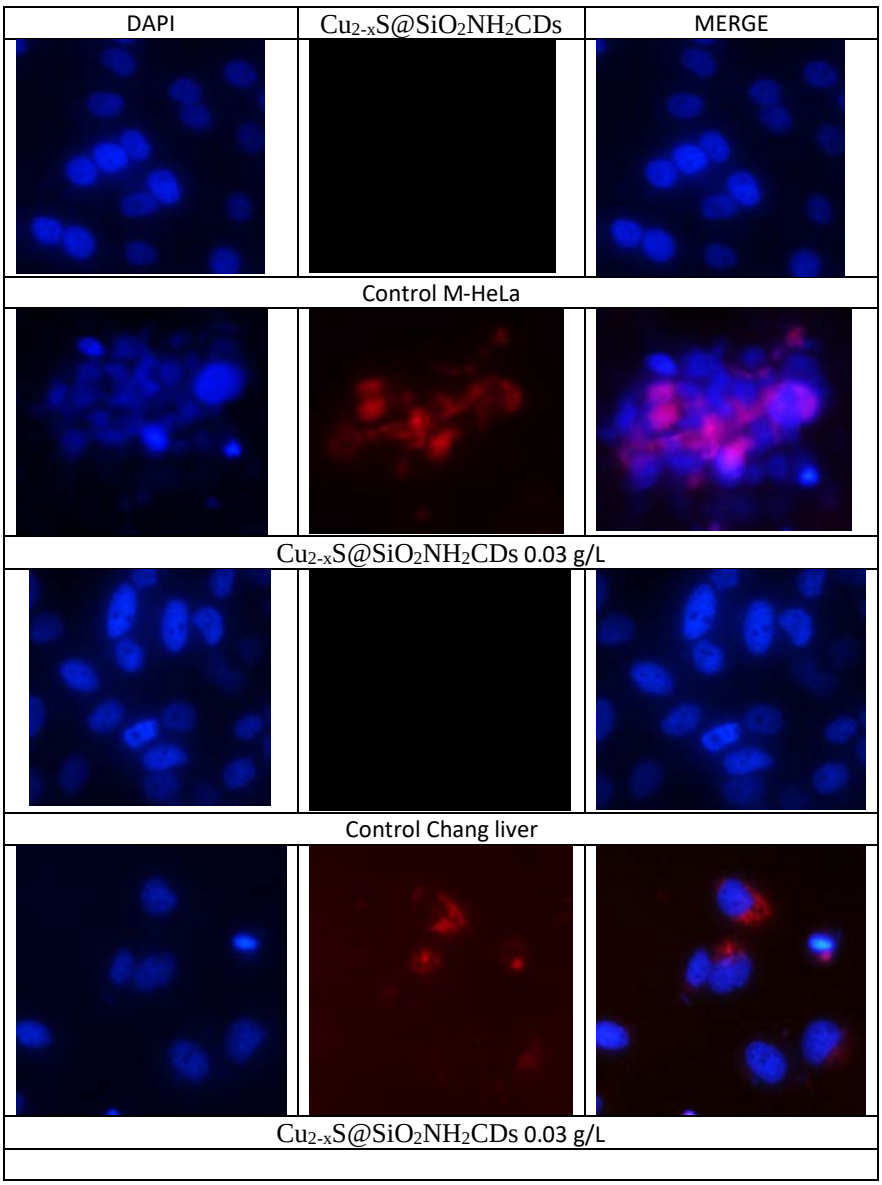
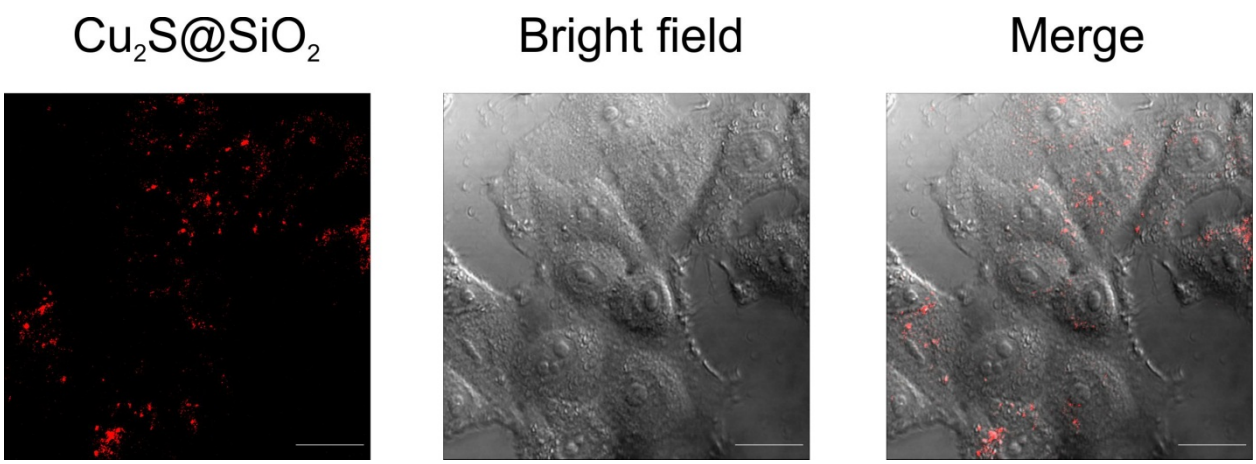
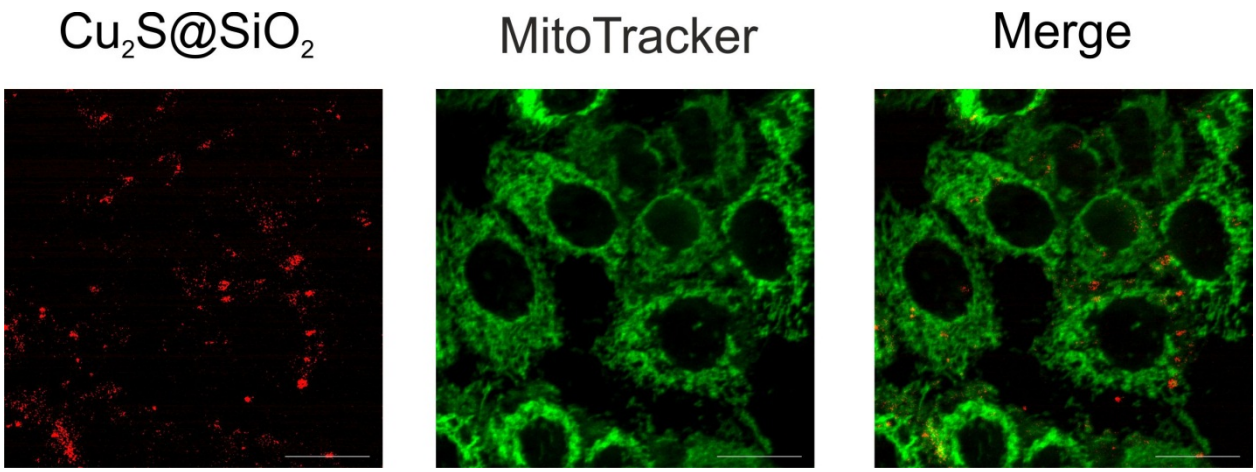


Fig. S5. Fluorescent microscopy images of Chang Liver and M-HeLa cells incubated with $Cu_{2-x}S@SiO_2$-NH_2-CDs and DAPI.





Scale Bar=20 μM

Fig. S6. Confocal microscopy images of the M-HeLa cells incubated with $Cu_{2-x}S@SiO_2$-NH_2-CDs (0.03 $g \cdot L^{-1}$) and MitoTracker.

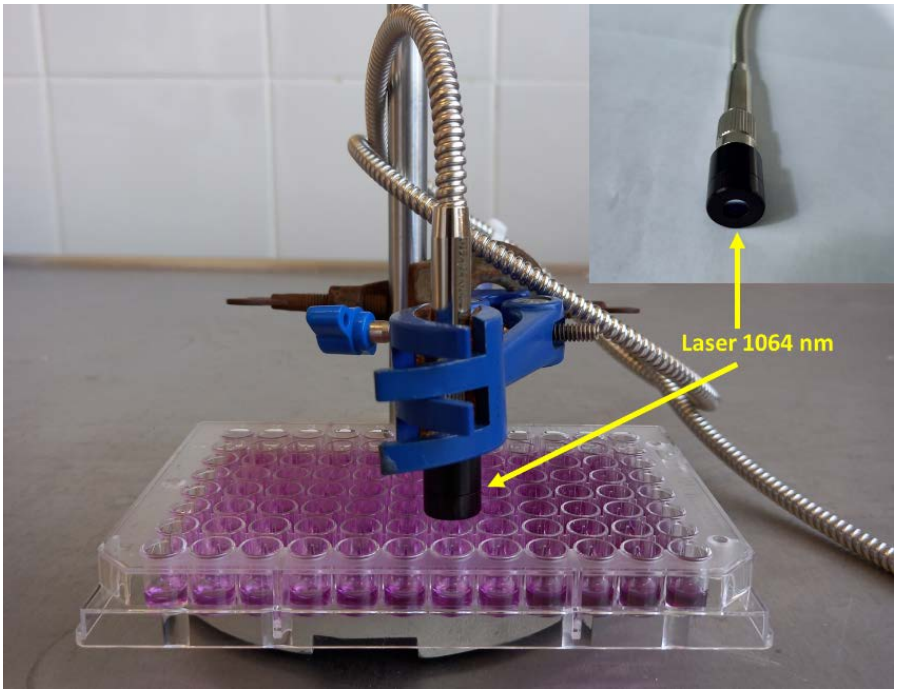


Fig. S7. Photo of the device which has been used for irradiation-induced cytotoxicity studies.