Electronic Supplementary Information (ESI)

Highly Monodisperse Beta-Cyclodextrin-Covellite Nanoparticles for Efficient Photothermal and Chemotherapy

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Experimental section

1. Chemicals: sulfur powder, ethanol, chloroform, tetrahydrofuran, ascorbic acid and sodium borohydride were purchased from Nanjing Chemical Reagent Co., Ltd. Sodium hydroxide (NaOH), 1-octadecene, oleic acid, Poly(isobutylene-alt-maleic anhydride) (Mw~6000 Da), dodecylamine and oleylamine were obtained from Sigma-Aldrich. Gold nanorods (Au NRs) were synthesized accroding to a previous reported method[1] and dispersed in 1 mM CTAB solution to avoid the aggregation under laser irradiation. Calcein AM, Propidium Iodide (PI) and 3-(4,5-dimethylthiozol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from KeyGEN BioTECH. Doxorubicin hydrochloride (DOX) was purchased from Sangon Biotech. 6-Deoxy-6-aminoethylamino-β-cyclodextrin (β-CD-NH_2) was purchased from Shandong Binzhou Zhiyuan Bio-Technology Co., Ltd. Copper acetylacetonate ($Cu(acac)_2$), Iron (III) acetylacetonate ($Fe(acac)_3$), Sodium oleate, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysulfosuccinimide sodium salt (NHS) were supplied by Aladdin Industrial Inc. Cyclo(Arg-Gly-Asp-D-Phe-Lys) peptide (c(RGDfK)) conjugated with adamantane (denoted as Ad-RGD; purity: 98%) was purchased from ChinaPeptides Co., Ltd. All the chemicals were used as received

without further purification. Millipore water (18.2 MΩ cm at 25 °C) was used throughout all experiments.

2. Characterization: Transmission electron microscopy (TEM) images were obtained using a JEOL JEM 1011 electron microscope at an acceleration voltage of 100 kV. UV-Vis spectra were recorded with a UV-3600 spectrophotometer (Shimadzu). X-ray powder diffraction (XRD) measurement was performed on a Shimadzu XRD-6000 with Cu Kα radiation (λ = 0.15418 nm) with a scanning rate of 2 deg/min. The photoacoustic imaging was obtained with a reported setup equipped with a 1064 nm nanosecond plused laser.[2] The fluorescence spectra were measured with a RF-5301PC fluorescence spectrometer. Zeta potentials were measured on a Malven Nano-Z instrument. Confocal laser scanning microscopy (CLSM) images were obtained using a Leica TCS SP5 microscope. Elemental composition of the copper sulfide nanoparticles was determined by an energy dispersive X-ray spectroscopy (EDX) system attached to a Hitachi S-4800 field emission scanning electron microscope. Hydrodynamic diameters were obtained through dynamic light scattering (DLS) with a 90 Plus Particle Size Analyzer (Brookhaven Instruments Corp.). FTIR spectra were recorded on a Nicolet 6700 spectrograph. The concentration of nanoparticles was all determined using absorption spectra with a mass extinction coefficient ~ 60 L $g^{-1}cm^{-1}$.

3. Synthesis of covellite (CuS) nanoparticles: In a typical procedure, 0.25 mmol $Cu(acac)_2$ was dissolved in 5 mL of oleylamine and 10 mL of 1-Octadecene at 55 ^oC under nitrogen flow, forming a green transparent solution. The sulfide precursor was prepared by dissolving 1 mmol sulfur powder in 10 mL of oleic acid at 130 ^oC for 30 minutes under nitrogen atmosphere. After cooling down to 55 ^oC, 5 mL of the sulfide precursor was added into the $Cu(acac)_2$ solution, and then the mixture was heated to 120 ^oC and mainteined at this temperature for 1 hour forming a dark green solution. After cooling down to the room temperature, the resulting CuS nanoparticles (CuS-OA) were precipitated with ethanol and washed with chloroform/ethanol, carbon

disulfide/ethanol and ethanol several times, and then re-dispersed in 5 mL chloroform and stored at 4 ^{o}C for further modifications.

4. Synthesis of amphiphilic polymer: the amphiphilic polymer with 75% of its andydride rings reacted with dodecylamine was synthesized according to the previous report.[3] Briefly, 1.542 g (10 mmol monomer) of poly(isobutylene-alt-maleic andydride) were placed in a round flask. Then, 7.5 mmol dodecylamine dissolved in 50 mL of anhydrous THF were added and stirred at 60 ^{o}C for 3 hours. After concentrate the reaction mixture roughly to one fifthe of the original volume by a rotavapor, the concentrated solution was further stirred at 60 ^{o}C overight. Finally, the obtained polymer was dried and re-dissolved in chloroform, the volume was adjusted to aound 12.5 mL, yield a monomer concentration of 0.8 M.

5. Synthesis of Polymer coated CuS NPs (CuS-COOH): 2 mL CuS nanoparticles solution (~ 4 mg/mL), 136 uL of amphiphilic polymer stock solution and 3 mL of $CHCl_3$ were mixed together and stirred for 20 minutes. The solvent was removed by rotary evaporation of yield a green CuS-polymer film. 6 mL of NaOH aqueous solution (0.1 M) was added and the solution was sonicated to obtain colloidal stable polymer grafted CuS NPs with abundant carboxyl groups (CuS-COOH). 8mL water was added thereafter to dilute the solution. The CuS solution was passed through a 0.22 um syringe filter and purified by ultracentrifugal filtration for 3 times. The obtained solution was further dialysis against PBS for 2 days.

6. Synthesis of β-cyclodextrin conjugated CuS nanoparticles (CuS-CD): CuS-COOH in 5 mL PBS (0.4 mg/mL) was mixed with 9 mg β-CD-NH_2 dissolved in 1 ml PBS, 2.8 mg EDC in 0.5 mL PBS and 7 mg NHS in 0.5 mL PBS was added later. The reaction was continued for 4 hours, and then the mixture was ultracentrifugated 3 times and dialysis agaist PBS for 24 hours. The final volume was adjusted to 5 mL.

7. Synthesis of RGD modified CuS nanoparticles (CuS-RGD): 3 ml of CuS-CD in PBS was mixed with 0.6 mL of 2 mg/mL Ad-RGD and stirred for 10 h. After that the

solution was unltrcentrifugated 3 times and dialysis for 24 h. The final product was adjusted to 3 mL in volume.

8. DOX loading on CuS-RGD nanoparticles (CuS-DOX): 1.5 mL of CuS-RGD was mixed with 0.1 mg DOX in 100 uL DI water and stirred for 12 h. After purification the obtained nanoparticles were dispersed in 1.5 mL PBS.

9. Measurement of the photothermal performance: An aqueous solution of CuS nanoparticles (300 uL) with different concentrations was added into a 96-well plate. Stabilized infrared fiber laser (980 nm or 808 nm) with a beam diameter of around 6 mm (LEO Photonics) were used to irradiate the nanoparticle dispersions. The temperature of the solution was measured with a TM902C thermodetector.

10. *In Vitro* photothermal ablation of cancer cells: HeLa cells (350,000 cells) were seeded onto a confocal dish (35 mm) at 37 ^{o}C with 5% CO_2 in complete medium one day before the treatment. Then, cells were washed with PBS and incubated with nanoparticles with a concentration of 10 ug/mL for 4 hours. A 980 nm laser with a power density of 1.5 W/cm^2 were used to irradiate the cells. After 4 minutes exposion, the nanoparticles were removed and cells were further incubated for 12 h. The cells were than washed with PBS and stained with calcein AM and PI for CLSM imaging.

11. *In Vitro* drug release: Buffers with different pHs were added into the drug loaded nanoparticles (CuS-DOX) to make the DOX contration to be 5 ug/mL. The emission intensity of DOX in the suspension at 555 nm was monitored to reflect the drug release amount.

12. Intracellular drug release: HeLa cells were seeded in a 35 mm confocal dish over night. Then the culture medium was removed and DOX loaded nanoparticles in fresh culture medium with a DOX concentration of 5 ug/mL was added. After incubated for 2 h, the cells were washed with PBS and stained with Hoechst 33342 (2 ug/mL) for 15 min at 37 ^{o}C. After three times wash with PBS, CLSM observation was carried out after wash away the excess Hoechst 33342.

13. Cell Viability studies: HeLa cells were seeded in 96-well plates (1×10^5 cells/well) and cultured for 24 h before experiment. Then, the meida was removed and the cancer cells were incubated with nanoparticles with different concentrations for 3 hours. 980 nm laser with a power density of 1.5 W/cm^2 was used to irradiate the cells (4 min each well). After wash with PBS, fresh culture medium was added and the cells were further cultured for 24 h. After this, a standard MTT assay was applied to determine the cell viabilities. Absorbance intensity at 490 nm was determined with Varioskan flash multimode reader (Thermo Scientific). At least three replicates were done for each group.

Table S1. Mass extinction coefficient of the established nanomaterials in the literature.

Material	Mass extinction coefficient	Wavelength
Nano-rGO[4]	21.1	808 nm
GO[4]	5.94	808 nm
MoS_2[4]	29.8	808 nm
WS_2[4]	23.8	808 nm
FeS[4]	15.5	808 nm
$MoSe_2$[5]	17.4	785 nm
TiS_2[6]	26.8	808 nm
SnS[7]	16.2	808 nm
Bi_2S_3[8]	20.5	808 nm
Au Nanorod[9]	13.9	808 nm
Au Nanorod[10]	20.0	808 nm
Cu_9S_5[11]	7.2	980 nm
CuS[12]	13.5	1064 nm
CuS[13]	44.9	930 nm
This work	32.4	808 nm
This work	60.7	965 nm

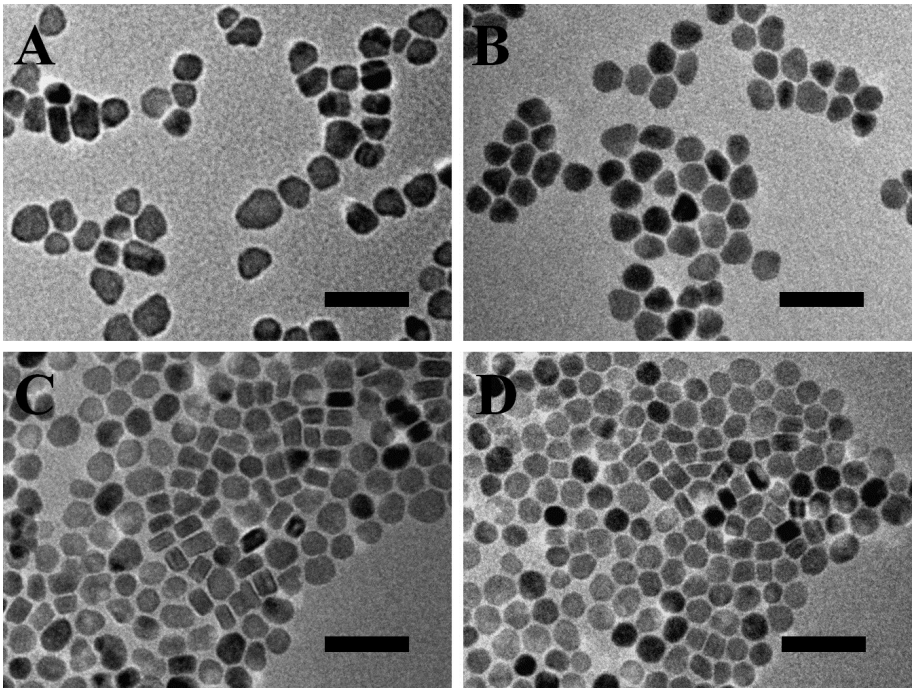

Fig. S1 Enlarged TEM images of copper sulfide nanoparticles synthesized with different Cu:S ratios, 1:0.5 (A); 1:1 (B);1:2 (C);1:4 (D). The scale bar is 50 nm. The copper sulfide nanoparticles synthesized with low Cu:S ratios (1:2 and 1:4) were found to be thick plate-like particles with an average thickness of ~9 nm.

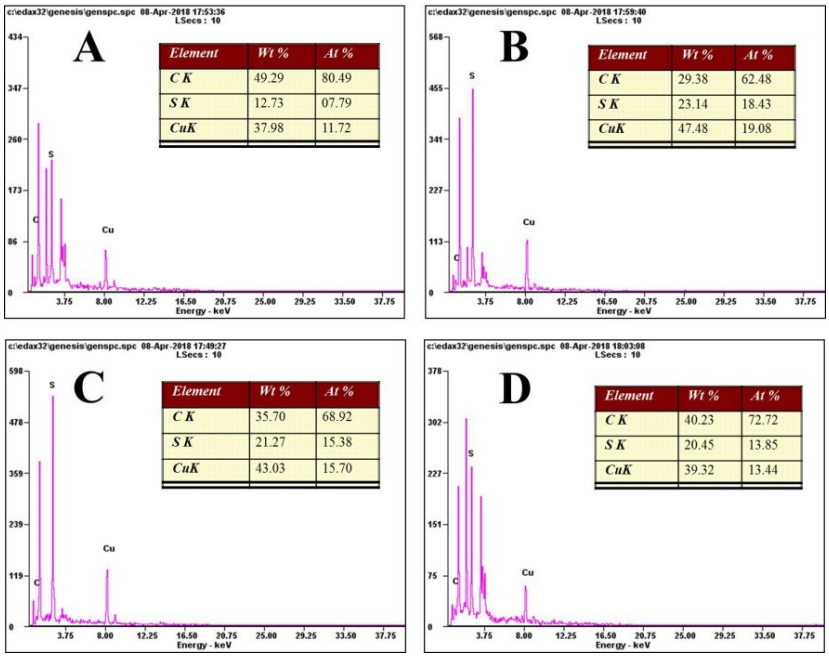

Fig. S2 Energy dispersive X-ray spectroscopy (EDX) of copper sulfide nanoparticles synthesized with different Cu:S ratios, 1:0.5 (A); 1:1 (B);1:2 (C);1:4 (D). The actual ratios were found to be 1:0.66, 1:0.96, 1:0.98 and 1:1.03 for the nanoparticles prepared with Cu:S ratio of 1:0.5, 1:1, 1:2 and 1:4 respectively, which is in accord with the XRD results.

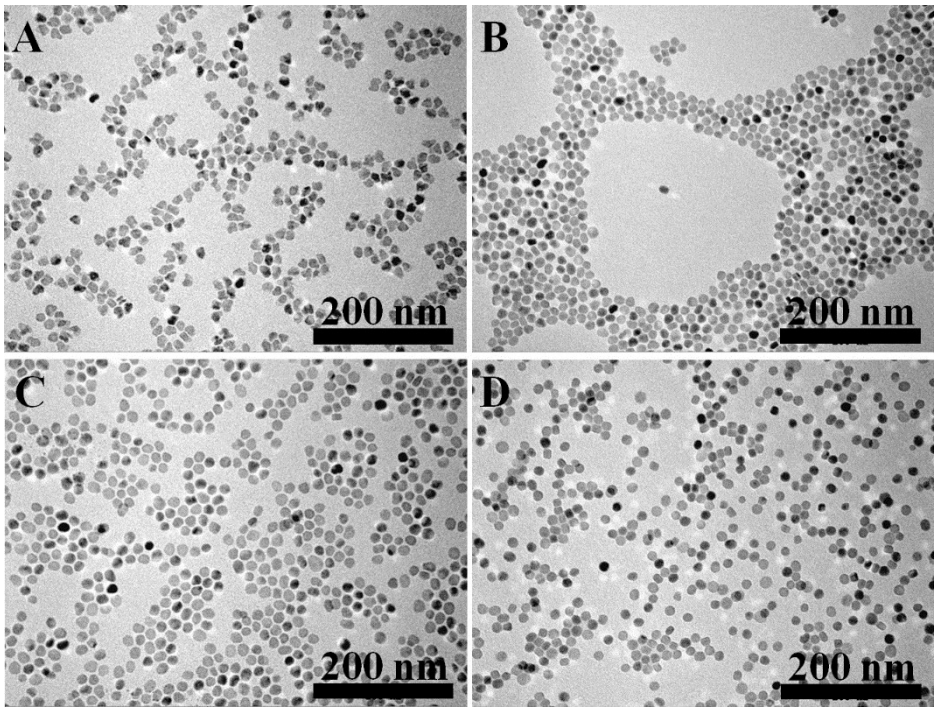

Fig. S3 TEM images of copper sulfide nanoparticles synthesized at different temperatures, 80 °C (A); 100 °C (B);120 °C (C);140 °C (D).

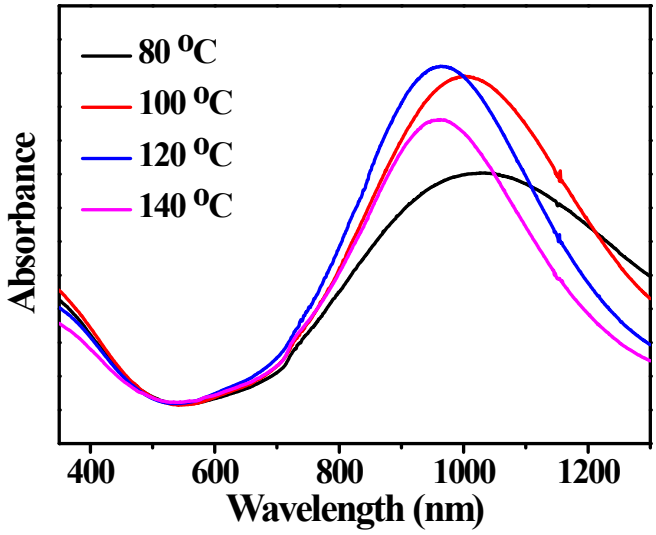

Fig. S4 Absorption spectra of copper sulfide nanoparticles synthesized at different temperatures.

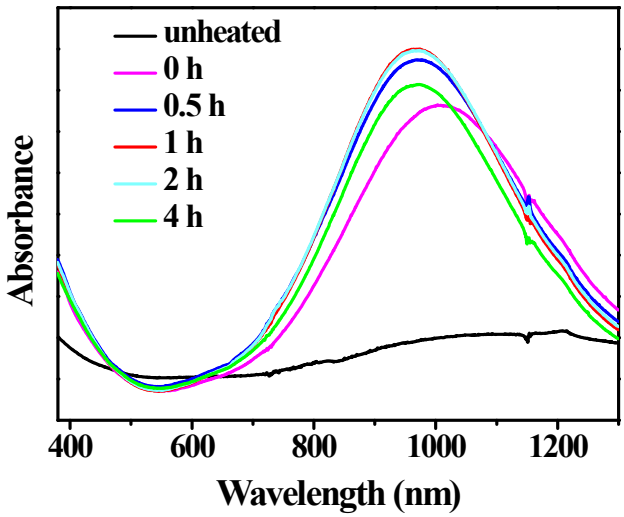

Fig. S5 Absorption spectra of copper sulfide nanoparticles synthesized with different reaction times. For 0 h sample, heating was stopped immediately after the temperature reached 120 °C.

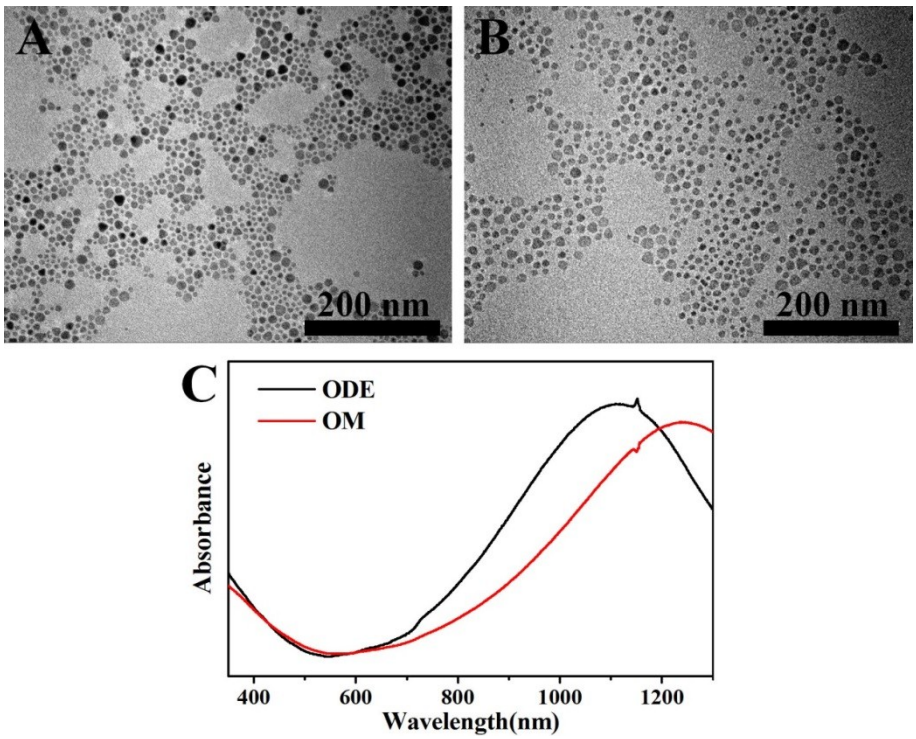

Fig. S6 Copper sulfide nanoparticles prepared with different organo-sulfur precursors, sulfur powder in 1-octadecene (A) and sulfur powder in oleylamine (B), and their corresponding abosorption spectra (C).

Table S2. Mass extinction coefficient of copper sulfide nanoparticles synthesized with different Cu:S ratios

Cu:S ratio	1:0.5	1:1	1:2	1:4
Mass Extinction Coefficient ($Lg^{-1}cm^{-1}$)	21.5	39.6	60.7	60.9

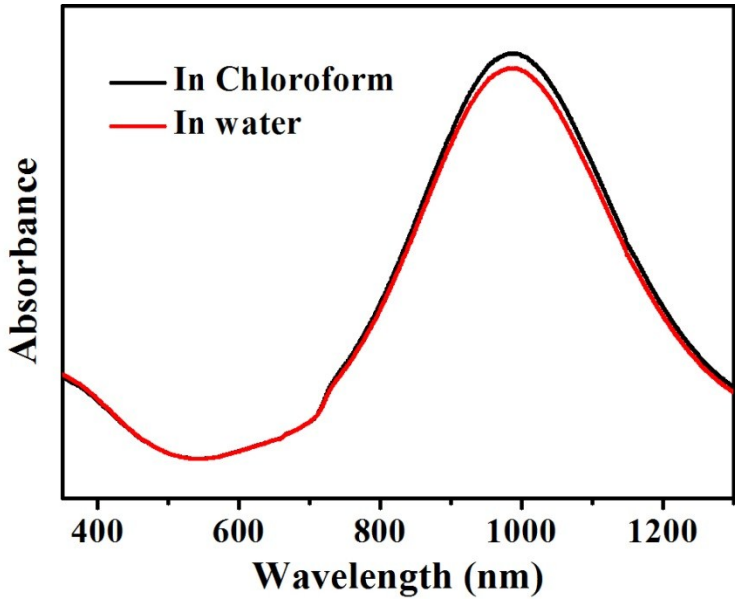

Fig. S7 Absorption spectra of CuS nanoparticles before and after phase transfer. The spectra of CuS nanoparticles in water was acquired before purification.

Table S3. Zeta potential of CuS nanoparticles with different surface modifications.

Sample	Test 1 (mV)	Test 2 (mV)	Test 3 (mV)	Average (mV)
CuS-COOH	-32.1	-34.2	-29.6	-32±2.3
CuS-CD	-20.4	-21.4	-20.1	-20.6±0.68
CuS-RGD	-22	-24.2	-24.7	-23.6±1.4
CuS-DOX	-15.2	-15.3	-16.3	-15.7±0.78

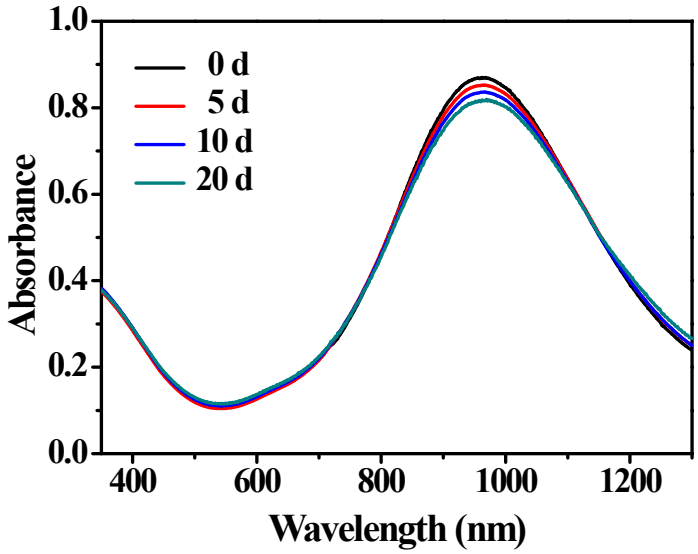

Fig. S8 Absorption spectra of amphiphilic polymer modified covellite nanoparticles (CuS-COOH) stored for different period of times. The decrease of the absorbance was less than 8% over 20 days storage.

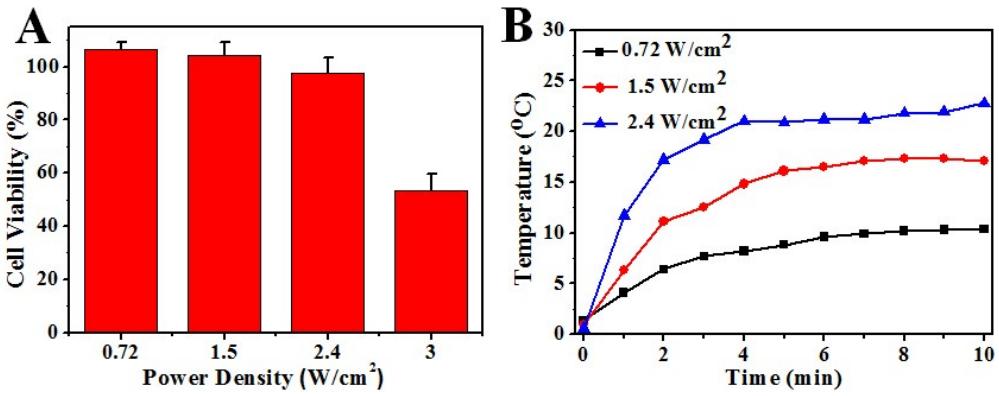

Fig. S9 (A) Cell viabilities of HeLa cells irradiated with a 980 nm laser for 4 min at different power densities. (B) The temperature separation between CuS-COOH (16.7 ug/mL) and water irradiated with 980 nm later with different power densities.

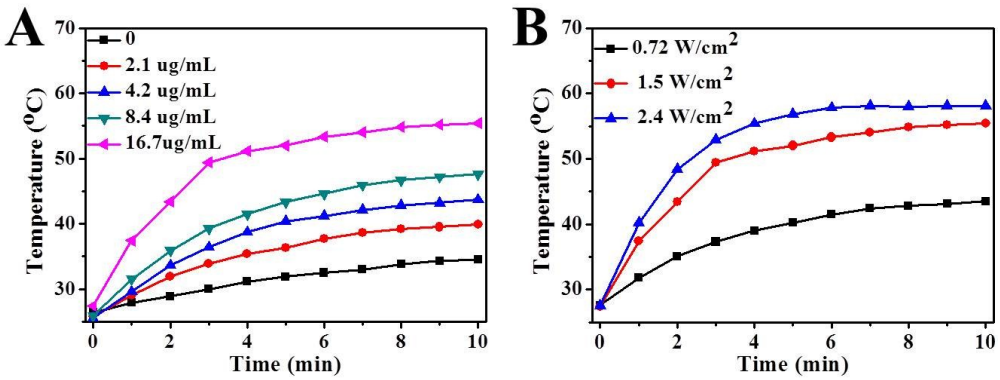

Fig. S10 Temperature elevation of covellite nanoparticle solutions after irradiated with 808 nm NIR laser (1.5 W/cm^2) with different concentrations (A) and laser power densities (B).

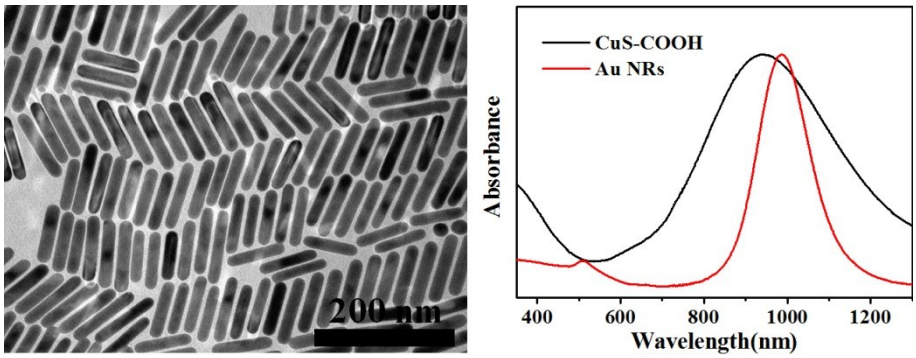

Fig. S11 TEM image (left) of as-prepared Au NRs. The absorption spectra (right) of CuS nanoparticles and Au NRs.

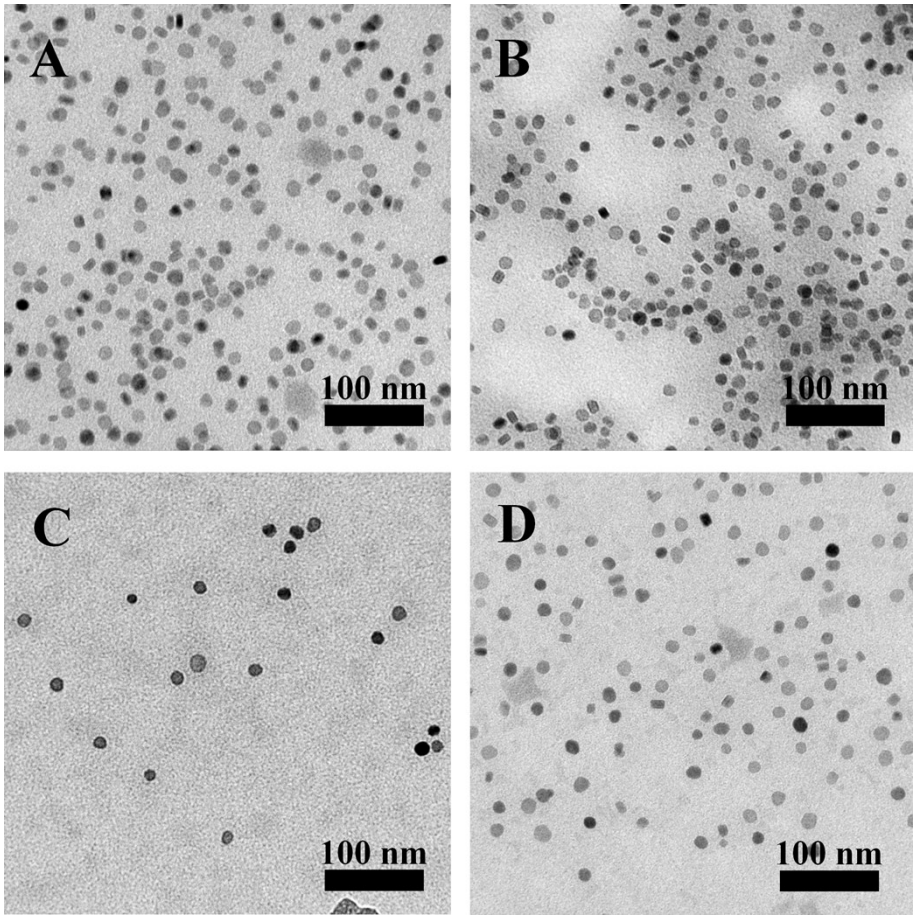

Fig. S12 TEM images of copper sulfide nanoparticles with different surface modifications, CuS-COOH (A); CuS-CD (B);CuS-RGD (C);CuS-DOX (D). All the nanoparticles maintain their morphology and monodispersity during the modification process, no aggregation was formed.

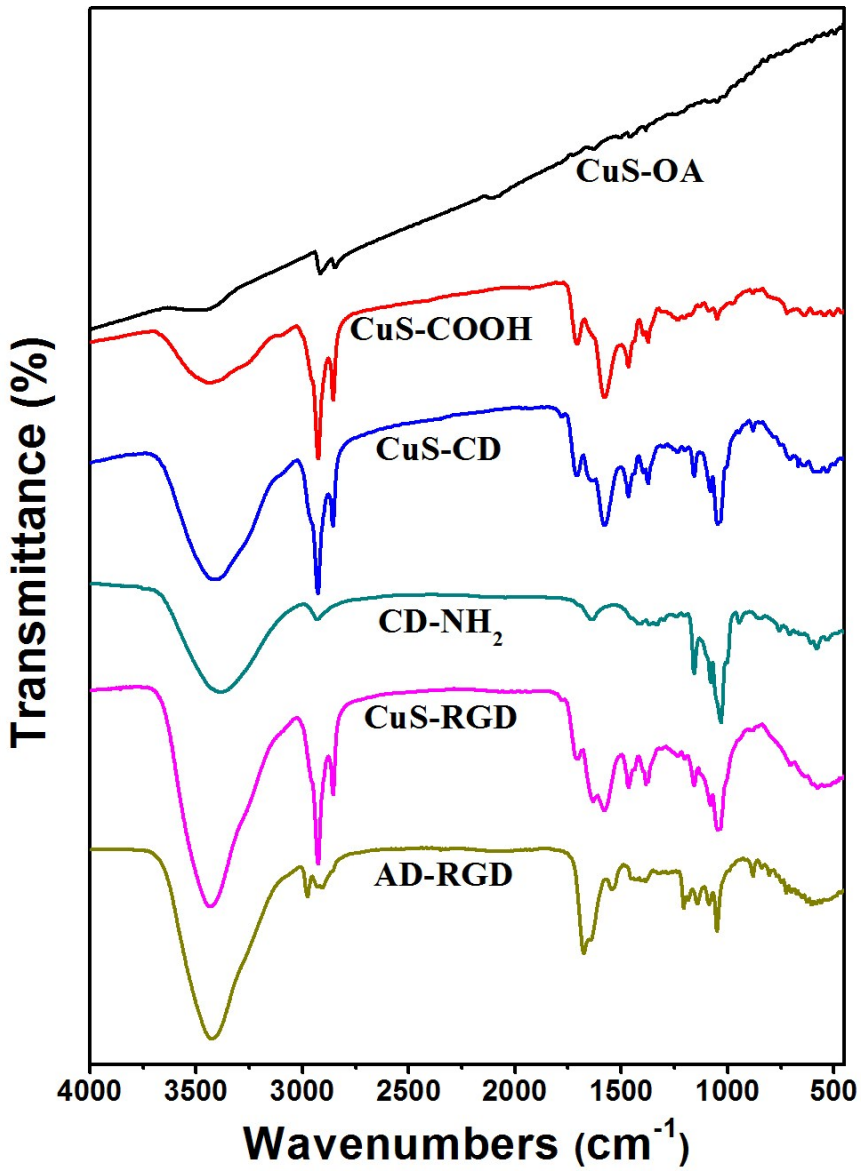

Fig. S13 FTIR spectra of CuS nanoparticles with different surface modifications. Strong absorption peaks of CD-NH_2 at 1035, 1080 and 1155 cm^{-1} appear for CuS-CD nanoparticles, which demonstrates the efficient conjugation of CD on CuS-COOH nanoparticles. After the modification of Ad-RGD, CuS-RGD nanoparticles exhibits a increased absorption band around 1635 cm^{-1} arising from the amide I band in the peptide,[14] which proves the sucessful functionalization of RGD.

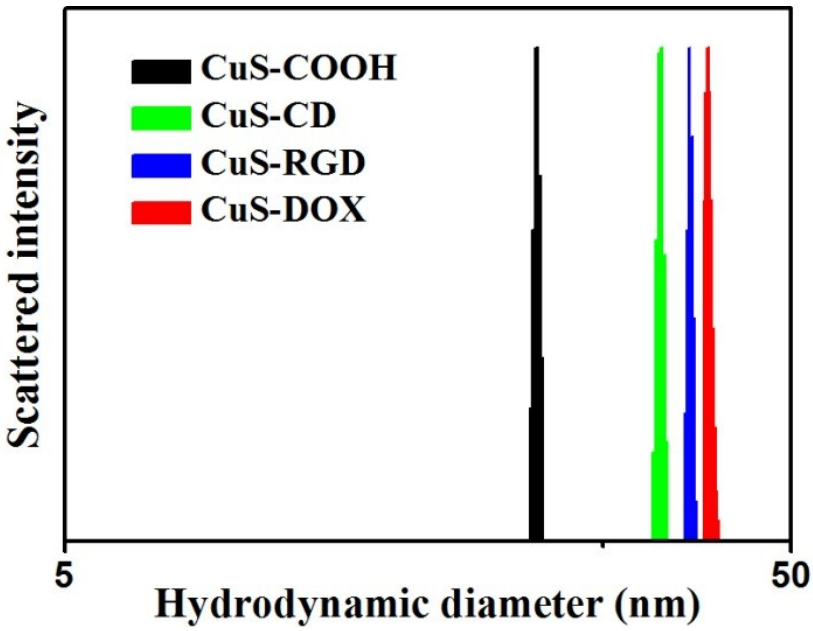

Fig. S14 Hydrodynamic diameters of CuS nanoparticles during the modifications. The hydrodynamic diameters were found to be 22.7, 33.1, 36.2 and 38.2 nm for CuS-COOH, CuS-CD, CuS-RGD and CuS-DOX nanoparticles respectively. All of the nanoparticles exhibited excellent colloidal stability and narrow size distributions in aqueous solution. Note that the modification of beta-cyclodextrin is a crucial step, too many EDC can lead to the aggregation and even precipitation of the nanoparticles.

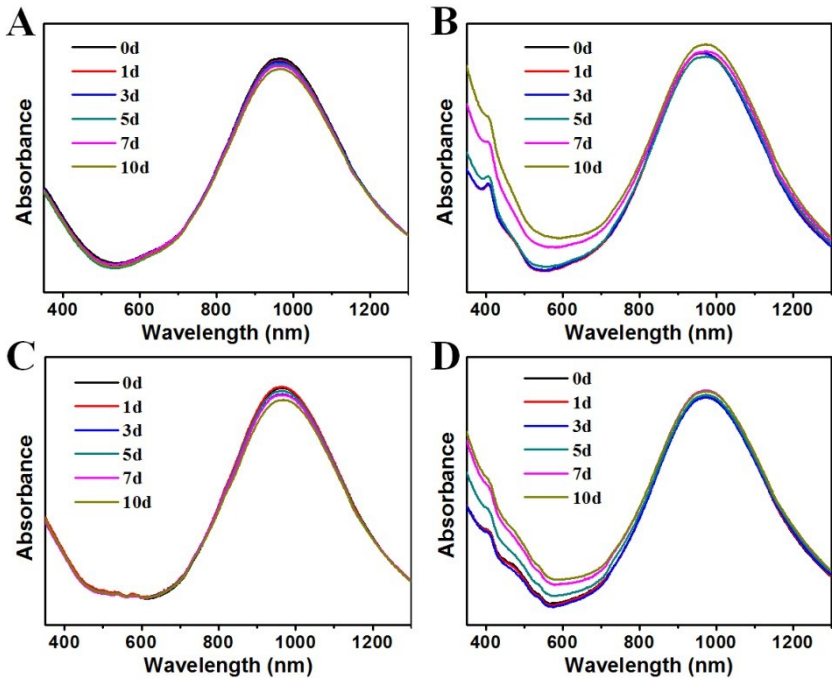

Fig. S15 Absorption spectra of CuS-RGD and CuS-DOX stored in PBS and 10% fetal bovine serum (FBS) for different period of times, CuS-RGD in PBS(A); CuS-RGD in FBS (B); CuS-DOX in PBS (C); CuS-DOX in FBS (D). The decrease of the absorbance of the nanoparticles around 965 nm was less than 5% over 10 days storage in PBS. The absorbance of nanoparticles around 965 nm in FBS slightly decreased in the first 3-5 days, then become slight increased in 7-10 days accompanied by the obviously increased absorbance aournd 400 nm. Since the absorption around 400 nm comes from FBS, the increased absorbance may due to the metamorphism of FBS after long time storage. The remain of the absorbance of CuS nanoparticles indicates their good stability in physiological solutions, (i) CuS nanoparticles do not decompose in physiological solutions; (ii) CuS nanoparticles remain colloidal stable in physiological solutions.

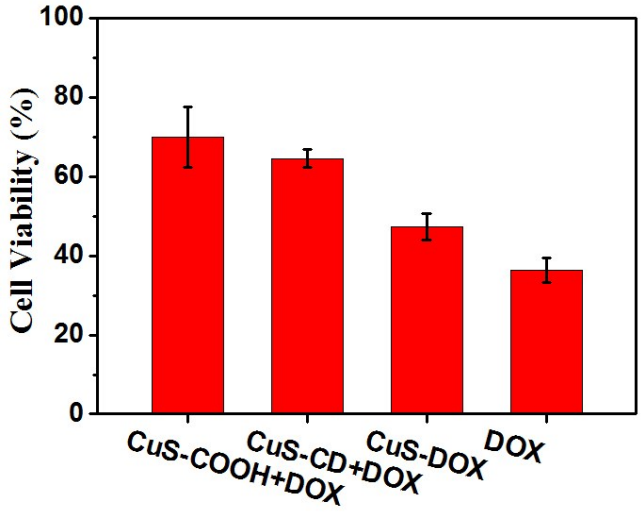

Fig. S16 The Cell viabilities of free DOX and DOX loaded CuS nanoparticles at an equivalent DOX concentration of 2.5 μg/mL. Higher cytotoxicity achieved with RGD modified CuS nanoparticles comparing with COOH and CD modified ones, indicates the positive effect on the therapeutic efficacy of target ligands. Free DOX shows highest cytotoxicity due their positive charge, however this property was not favored for *in vivo* applications.[15-17]

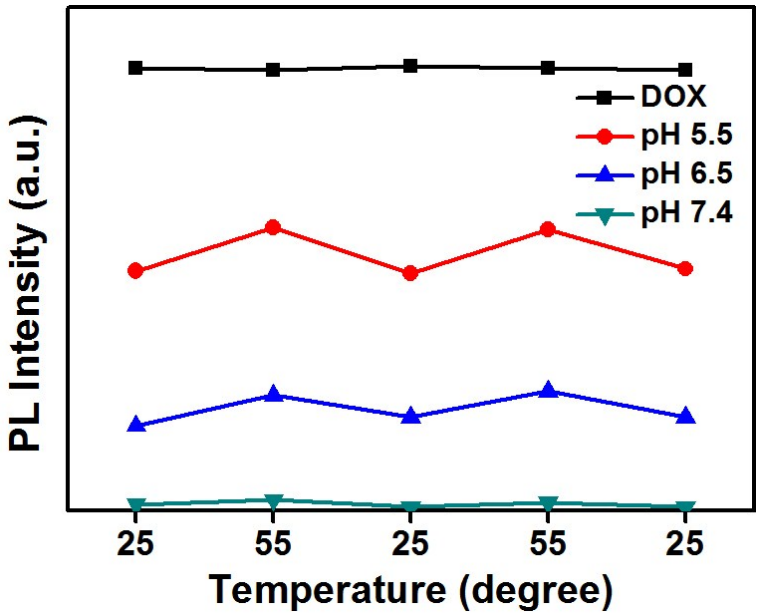

Fig. S17 The influence of temperature on the emission intensity (550 nm) of free DOX (pH 7.4) and CuS-DOX nanoparticles at different pHs.

Reference:

[1] X. C. Ye, C. Zheng, J. Chen, Y. Z. Gao, C. B. Murray, *Nano Lett.* , 2013, **13**, 765-771.

[2] X. Zhang, X. Q. Qian, C. Tao, X. J. Liu, *Ultrasound in Medcine and Biology*, 2018, **44**, 1110-1118.

[3] C. A. J. Lin, R. A. Sperling, J. K. Li, T. Y. Yang, P. Y. Li, M. Zanella, W. H. Chang, W. G. J. Parak, *Small*, 2008, **4**, 334-341.

[4] K. Yang, G. B. Yang, L. Chen, L. Cheng, L. Wang, C. C. Ge, Z. Liu, *Biomaterials*, 2015, **38**, 1-9.

[5] L. H. Yuwen, J. J. Zhou, Y. Q. Zhang, Q. Zhang, J. Y. Shan, Z. M. Luo, L. X. Weng, Z. G. Teng, L. H. Wang, *Nanoscale*, 2016, **8**, 2720-2726.

[6] X. X. Qian, S. D. Shen, T. Liu, L. Cheng, Z. Liu, *Nanoscale*, 2015, **7**, 6380-6387.

[7] Q. L. Ren, B. Li, Z. Y. Peng, G. J. He, W. L. Zhang, G. Q. Guan, X. J. Huang, Z. Y. Xiao, L. J. Liao, Y. S. Pan, X. J. Yang, R. J. Zou, J. Q. Hu, *New J. Chem.* , 2016, **40**, 4464-4467.

[8] Z. Y. Xiao, C. T. Xu, X. H. Jiang, W. L. Zhang, Y. X. Peng, R. J. Zou, X. J. Huang, Q. Liu, Z. Y. Qin, J. Q. Hu, *Nano Res*, 2016, **9**, 1934-1947.

[9] J. T. Robinson, K. Welsher, S. M. Tabakman, S. P. Sherlock, H. L. Wang, R. Luong, H. J. Dai, *Nano Res*, 2010, **3**, 779-793.

[10] Y. Lyu, C. Xie, S. A. Chechetka, E. Miyako, K. Y. Pu, *J. Am. Chem. Soc.* , 2016, **138**, 9049-9052.

[11] Q. W. Tian, F. R. Jiang, R. J. Zou, Q. Liu, Z. G. Chen, M. F. Zhu, S. P. Yang, J. L. Wang, J. H. Wang, J. Q. Hu, *Acs Nano*, 2011, **5**, 9761-9771.

[12] G. Ku, M. Zhou, S. L. Song, Q. Huang, J. Hazle, C. Li, *Acs Nano*, 2012, **6**, 7489-7496.

[13] M. Zhou, R. Zhang, M. A. Huang, W. Lu, S. L. Song, M. P. Melancon, M. Tian, D. Liang, C. Li, *J. Am. Chem. Soc.* , 2010, **132**, 15351-15358.

[14] C. Park, K. Lee, C. Kim, *Angew. Chem. Int. Ed.*, 2009, **48**, 1275-1278.

[15] Q. Zhang, F. Liu, K. T. Nguyen, X. Ma, X. J. Wang, B. G. Xing, Y. L. Zhao, *Adv. Funct. Mater.* , 2012, **22**, 5144-5156.

[16] Q. Zhang, X. L. Wang, P. Z. Li, K. T. Nguyen, X. J. Wang, Z. Luo, H. C. Zhang, N. S. Tan, Y. L. Zhao, *Adv. Funct. Mater.* , 2014, **24**, 2450-2461.

[17] N. Kamaly, Z. Y. Xiao, P. M. Valencia, A. F. Radovic-Moreno, O. C. Farokhzad, *Chem. Soc. Rev.* , 2012, **41**, 2971-3010.