Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Electronic Supplementary Information

Using host-guest interactions at the interface of quantum dots to load drug molecules for a biocompatible, safe and effective chemo-photodynamic therapy against cancer

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S1. Experimental Section

Characterization: Transmission electron microscope (TEM) images of the nanoparticles were obtained by JEOL-2100 (JEOL, Japan) equipment, which was operated at 80 kV. The particle size and size distribution were measured by dynamic light scattering (DLS) using a zeta potential and size analyzer (Nano-ZS, Malvern, UK) with a detection angle of scattered light at 173°. ^{1}H NMR and 2D NOESY spectra were performed on a Bruker Advance DMX 600 MHz spectrometer. The Ag concentration of Ag_2S-DOX-CP6 were determined utilizing inductively coupled plasma optical emission spectrometry (ICP-OES; Optima 2100DV instrument, PerkinElmer, Waltham, MA, USA). Confocal Laser scanning microscopy (CLSM) of Zeiss LSM 780 were used to record cell fluorescence images. The apoptosis assay was then analyzed using flow cytometry (BD LSRFortessas). Each assay was performed in triplicate. The fluorescence imaging of mice was tested using IVIS Lumina XRMS In Vivo Imaging System (PerkinElmer) with the excitation channel of 710 nm and emission channel of 790 nm.

The drug loading and release: To calculate the DOX encapsulation efficiency (EE%) and loading efficiency (LE%), the supernatant was collected after centrifugation of Ag_2S-DOX-CP6, and the residual DOX content was determined using the calibration curve of DOX standard solutions by the UV-vis absorption peak at 480 nm. The EE% and LE% of DOX in Ag_2S-DOX-CP6 were calculated as follows:

$$EE\% = \frac{weight\ of\ initial\ DOX - weight\ of\ residual\ DOX}{weight\ of\ initial\ DOX} \times 100\%$$

$$LE\% = \frac{weight\ of\ initial\ DOX - weight\ of\ residual\ DOX}{weight\ of\ Ag_2S - DOX - CP6} \times 100\%$$

To investigate the drug release behavior of Ag_2S-DOX-CP6, the prepared Ag_2S-DOX-CP6 were dispersed into 1 mL of PBS at pH 7.4 and 5.5, and then transferred into dialysis bags (3500 Da). Then the dialysis bags were placed into beakers containing 49 mL of PBS with pH 7.4 and 5.5, and the solutions were gently stirred under dark conditions. At predetermined time intervals, 1 mL of PBS was removed for UV-vis measurement to determine the amount of released DOX, and 1 mL of fresh PBS (pH 7.4 or 5.5) was returned to the original solution simultaneously.

To evaluate the photo-induced drug release behavior of Ag_2S-DOX-CP6, 1 mL of Ag_2S-DOX-CP6 dispersed in PBS (pH 7.4 and 5.5) was added into a cuvette and then irradiated under an 808 nm NIR laser at a power density of 2.0 W/cm^2 for different time (0-20 min). After irradiation, the supernatant

was collected by centrifugation (8000 rpm, 5 min), and the amount of released DOX was determined from the UV-vis absorption peak at 480 nm by calibration curve of DOX.

The detection of extracellular and intracellular ROS generation: To evaluate extracellular ROS generation of PDT for Ag_2S-DOX-CP6, 2,7-dichlorodi-hydrofluorescein diacetate (DCFH-DA) was utilized as a fluorescence probe to detect the generated ROS.[S1] In a typical procedure, 2,7-dichlorodi-hydrofluorescein (DCFH) was obtained by mixing DCFH-DA in methanol (0.5 mL, 1 mM) with NaOH (2 mL, 0.01 M) and stirring rigorously for 30 min in dark at room temperature. Then 10 mL of PBS (pH 7.4) was added to adjust the pH of above solution to neutral. Then 0.1 mL of as-prepared DCFH solution was mixed with 0.05 mL of Ag_2S-DOX-CP6 solutions (1 mg/mL) and 1.85 mL PBS, and then transferred into a quartz cell. The reaction solution was irradiated by 808 nm NIR laser at the optical power density of 1.0 W/cm^2 for different time (0-20 min) in dark. The photoluminescence spectra of the dispersions were measured every 5 min, and DCFH solution absence of Ag_2S QDs was treated as a control under the same measuring conditions. In addtion, ROS of $\cdot OH$, H_2O_2, $\cdot O^{2-}$ and $^1O_2$ were also detected using coumarin-3carboxylic acid (CCA), *p*-hydroxyphenylacetic acid (HPA), nitro blue tetrazorium (NBT) and 1,3-diphenylisobenzofuran (DPBF) for these samples under NIR laser 1.0 W/cm^2 for different time (0-20 min).

For the intracellular ROS detection of Ag_2S-DOX-CP6 under the irradiation by an 808 nm NIR laser using flow cytometry, human breast cancer cells (MCF-7 cells) were incubated with free DOX, Ag_2S-CP6 and Ag_2S-DOX-CP6 (5 ug/mL) in 24-well plates for 4 h, PBS was utilized to wash away the free nanoparticles, and fresh culture media containing DCFH (20 μM) was placed and incubated for another 30 min in 37 °C, 5% CO_2 incubator. The cells were washed three times with PBS to remove the free DCFH. After irradiated with 808 nm light (1.0 W/cm^2 for 1 min). Then MCF-7 cells were collected and dispersed in FlowTubes™ for flow cytometry (1×10^6 cells/mL). The cells with DCF were excited at 488 nm to collect the fluorescence by flow cytometry.

Meanwhile, CLSM was used to observe the cellular ROS generation of Ag_2S-DOX-CP6 under 808 nm light. Briefly, MCF-7 cells were incubated with free DOX, Ag_2S-CP6, or Ag_2S-DOX-CP6 (DOX concentration of 5 μg/mL) for 4 h. The cells were washed with PBS, and DCFH-DA (20 μM) was added for another 30 min incubation. After washed with PBS and irradiated under 808 nm light (1.0 W/cm^2, 1 min), the cells could be tested using CLSM under excitation of 488 nm laser.

***In vitro* biocompatibility:** MCF-7 cells were cultured in DMEM supplemented with 10 wt% fetal bovine serum (FBS), 1% GlutaMAX™ Supplement, 100 units/mL of penicillin and 100 mg/mL of streptomycin. The cells were incubated at 37 °C in a humidified atmosphere containing 5% of CO_2.

To evaluate the *in vitro* cytotoxicity of self-assembled nanocarriers, MTT assays were performed on the MCF-7 cells. 100 μL of MCF-7 cells were seeded in a 96-well plate at a density of 1 × 10^5 cells per mL, and then incubated for 24 h. 100 μL of Ag_2S QDs and Ag_2S-CP6 (0-50 μg/mL) of the culture medium were added and incubated for another 24 h. 10 μL of MTT (5 mg/mL) was added to each well. After an additional 4 h incubation, the medium and MTT were removed, and the MTT-formazan crystals in each well were dissolved in 100 μL of dimethyl sulfoxide (DMSO). The absorbance of the suspension was recorded using a microplate reader (Thermo MultiskanFC, USA) at a wavelength of 570 nm and 620 nm.

Cellular uptake evaluation: Flow cytometry was utilized to evaluate the cellular uptake of free DOX and Ag_2S-DOX-CP6. 2 mL of MCF-7 cells were seeded in a culture dish at a density of 5 × 10^5 per mL, and incubated at 37 °C in 5% CO_2 for 24 h. Then, the culture medium was replaced with 2 mL of fresh culture containing free DOX (5 μg/mL) or Ag_2S-DOX-CP6 (50 μg/mL, and the DOX concentration in nanocarriers was 5 μg/mL). After incubated for another 4 h, the cells were washed three times with PBS to remove the free nanoparticles and the free DOX. Then MCF-7 cells were collected and dispersed in FlowTubes™ for flow cytometry (cell concentration is 1 × 10^6 cells/mL). The fluorescence channel was chosen PerCP for DOX uptake and cell samples were tested by flow cytometry.

In addition, CLSM was used to observe the cellular uptake of free DOX and Ag_2S-DOX-CP6. 2 mL of MCF-7 cells were seeded in a culture dish at a density of 5 × 10^5 per mL, and incubated at 37 °C in 5% CO_2 for 24 h. Then, the culture medium was replaced with 2 mL of fresh culture containing free DOX (5 μg/mL) or Ag_2S-DOX-CP6 (50 μg/mL). After incubated for another certain times (10, 30 and 60 min), the cells were washed three times with PBS to remove the free nanoparticles and the free DOX. Then the cells were treated with LysoTracker™ Deep Red (50 nM) and Hoechst 33342 (1 μg/mL) at room temperature to stain the cytoskeleton and nucleus. Hoechst 33342, DOX and LysoTracker™ Deep Red were excited at 405, 488 and 635 nm, respectively, and the fluorescence images at emission wavelengths of 430-490, 550-640 and 650-720 nm were obtained using Zeiss LSM 780.

***In vitro* chemotherapy:** The chemotherapy effect of Ag_2S-DOX-CP6 for breast cancer was evaluated by cell viability assay *in vitro*. 100 μL of MCF-7 cells were seeded in a 96-well plate at a density of 1 × 10^5 cells per mL, and incubated for 24 h. 100 μL of free DOX and Ag_2S-DOX-CP6

nanoparticles (DOX concentration is 0-4 μg/mL) dispersed in the culture medium were added and incubated for another 24 h. 10 μL of MTT (5 mg/mL) was added to each well. After an additional 4 h incubation, the medium and MTT were removed, and the MTT-formazan crystals in each well were dissolved in 100 μL of DMSO. The absorbance of the suspension was recorded using a microplate reader at a wavelength of 570 nm and 620 nm to calculate the cell viabilities.

***In vitro* PDT effects:** The chemo-photodynamic synergistic therapeutic effect of Ag_2S-DOX-CP6 for breast cancer was evaluated by MTT assay similar to the method above. The difference were nanoparticles of Ag_2S-CP6 and Ag_2S-DOX-CP6 (DOX concentration of 0-2 μg/mL) and irradiating by an 808 nm NIR laser (2.0 W/cm^2, 10 s for each well) after 4 h incubation, and continually cultured for another 20 h. Then the samples were recorded by microplate reader to calculate the cell viabilities.

In Vitro Dead/Live Imaging. The anti-cancer effect of co-delivery system was also evaluated by fluorescence imaging of dead/live cells. 2 mL of MCF-7 cells were seeded in 6-well plates at a density of 5×10^5 per mL for 24 h incubation. Then, the culture medium was replaced with 2 mL of fresh culture containing free DOX, Ag_2S-CP6, or Ag_2S-DOX-CP6 (DOX concentration of 5 μg/mL) for 4 h (the cultures without any drugs and nanoparticles were used as control groups). Then they were irradiated by an 808 nm laser for 1 min at power density of 1.0 W/cm^2. After 20 h incubation, MCF-7 cells were collected and washed with PBS to remove the free nanoparticles. Then the collected cells were stained using Calcein-AM/PI Cell Viability/Cytotoxicity Detection Kit and observed by CLSM.

Cell Apoptosis Assay: MCF-7 cells were planted into 12-well plates at a density of 1.0×10^5 cells per well and cultured for 24 h. Cells with no treatment were used as control, then the culture medium was replaced with free DOX and Ag_2S-DOX-CP6 nanoparticles at a DOX concentration of 2 μg/mL in culture medium. After 4 h incubation, the cells were harvested, washed, and treated with Dead Cell Apoptosis Kit with Annexin V FITC and PI. Finally, those cells were collected and analyzed using flow cytometry (BD LSRFortessas) for apoptosis assay. Each assay was performed in triplicate.

***In vivo* biocompatibility:** Male Institute of Cancer Research (ICR) mice (7-8 weeks old) and Female BALB/c-nu mice (5-6 weeks old) were purchased Nanjing Cavins Biotechnology Co., Ltd (Nanjing, China) and housed in a 12 h light/dark cycled facility with free access to food and water. All experiments were reviewed and approved by the Regional Ethics Committee for Animal Experiments at Ningbo University, China (permit no. SYXK (Zhe) 2019-0005).

Before the evaluation of treatment effect of Ag_2S-DOX-CP6 *in vivo*, the biocompatibility of self-assembled nanocarriers were studied on mice. Three groups of ICR mice (PBS, Free DOX, and Ag_2S-

DOX-CP6) were injected one time of 0.2 mL PBS, DOX (0.25 mg/mL in PBS), and Ag_2S-DOX-CP6 (2.5 mg/mL in PBS). On day 15, all mice were sacrificed, and subcutaneous major organs of heart, liver, spleen, lung and kidney were collected and stored in 4% paraformaldehyde at 4 °C.

***In vitro* fluorescence imaging.** Female BALB/c-nu mice (5-6weeks old) were implanted subcutaneously into the right leg with 1×10^7 MCF-7 cells in 0.1 mL PBS. When tumors grew to a certain volume of 200 mm^3, mice received intravenous injection of IR-783 and Ag_2S-DOX-CP6-783. Then the fluorescence distribution of co-delivery system could be observed at certain time points by IVIS Lumina LT *In Vivo* Imaging System (PerkinElmer), which was excited at wavelength of 700 nm and the emission light was collected using 790 nm filter. Next, after intravenous injection of certain time, mice were sacrificed, and subcutaneous tumors and major organs to distribution of Ag_2S-DOX-CP6 in tumor bearing mice.

***In vivo* therapy on tumor-bearing mice:** Female BALB/c-nu mice were implanted subcutaneously into the right leg with 1×10^7 MCF-7 cells in 0.1 mL PBS. Tumor growth were observed until the volume of subcutaneous tumors reached about 100 mm^3. Mice with tumors were randomly assigned to six groups (n = 4): PBS (0.2 mL sterilized PBS), PBS-808 (0.2 mL sterilized PBS with 808 nm irradiation), DOX (0.2 mL 0.25 mg/mL DOX in PBS), Ag_2S-DOX-CP6 (0.2 mL 2.5 mg/mL in PBS), Ag_2S-CP6-808 (0.2 mL 2.25 mg/mL with 808 nm irradiation) and Ag_2S-DOX-CP6-808 (0.2 mL 2.5 mg/mL with 808 nm irradiation). All mice received intravenous injection every 4 days for 12 consecutive days (3 times treatments). For groups of PBS-808, Ag_2S-CP6-808 and Ag_2S-DOX-CP6-808, mice would be irradiated by an 808 nm of NIR laser at 2.0 W/cm^2 for 5 min after 24 h of tail vein injection. The tumor volume was calculated according to the equation $V = A \times B^2 / 2$ (mm^3), where A represents the largest diameter and B represents the vertical diameter. When the tumor volume was larger than 1000 mm^3, the mouse could be considered dead induced by cancer. The body weight of each mouse was measured and recorded every 2 days for 16 consecutive days. On day 16, all mice were sacrificed, and subcutaneous tumors were collected. Before mice sacrificed, blood was collected using a standard saphenous vein blood collection technique for hematology analysis.

Hematoxylin-eosin (H&E) staining: The organs from each group were post-fixed in formaldehyde (4%), dehydrated, and embedded in paraffin. Subsequently, the specimens were cut cross-section at the intermediate part into 5 μm thick slices and stained with hematoxylin-eosin dyes.

Histopathology was observed under a phase-contrast microscope to test the toxicity of hybrid nanocarriers.

Statistical analysis: Differences between these groups were statistically analyzed using the paired Student's t-test. A statistically significant difference was reported if $p < 0.05$ or less (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

S2. Figures

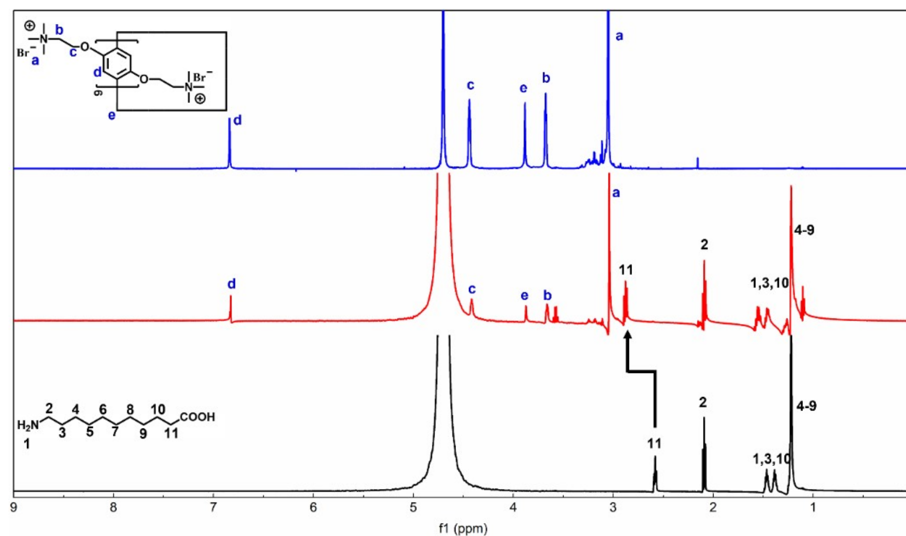

Fig. S1 The ^{1}H NMR (600 MHz) spectra of 11-AUA (black line), CP6⊃11-AUA (red line) and 11-AUA (blue line) in D_2O.

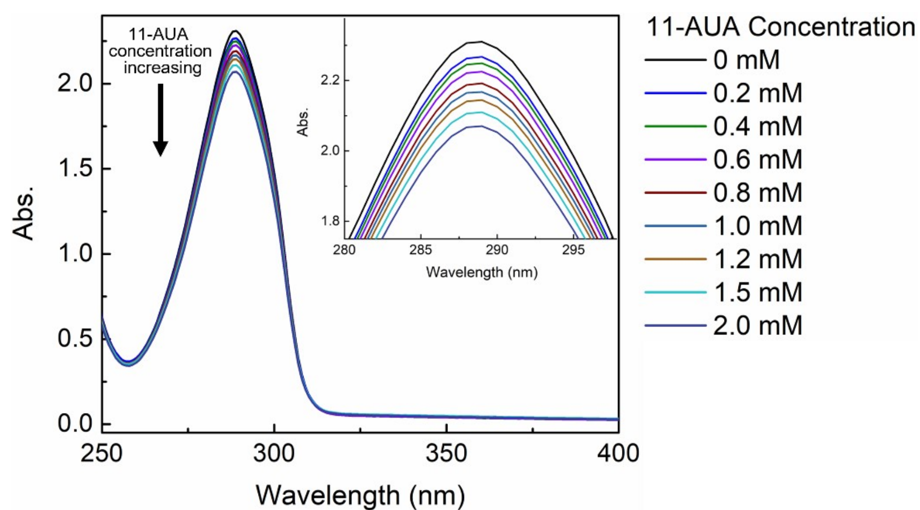

Fig. S2 UV-Vis spectra of CP6 (0.1 mM) in a phosphate buffer solution at room temperature with different concentrations of 11-AUA (0-2 mM).

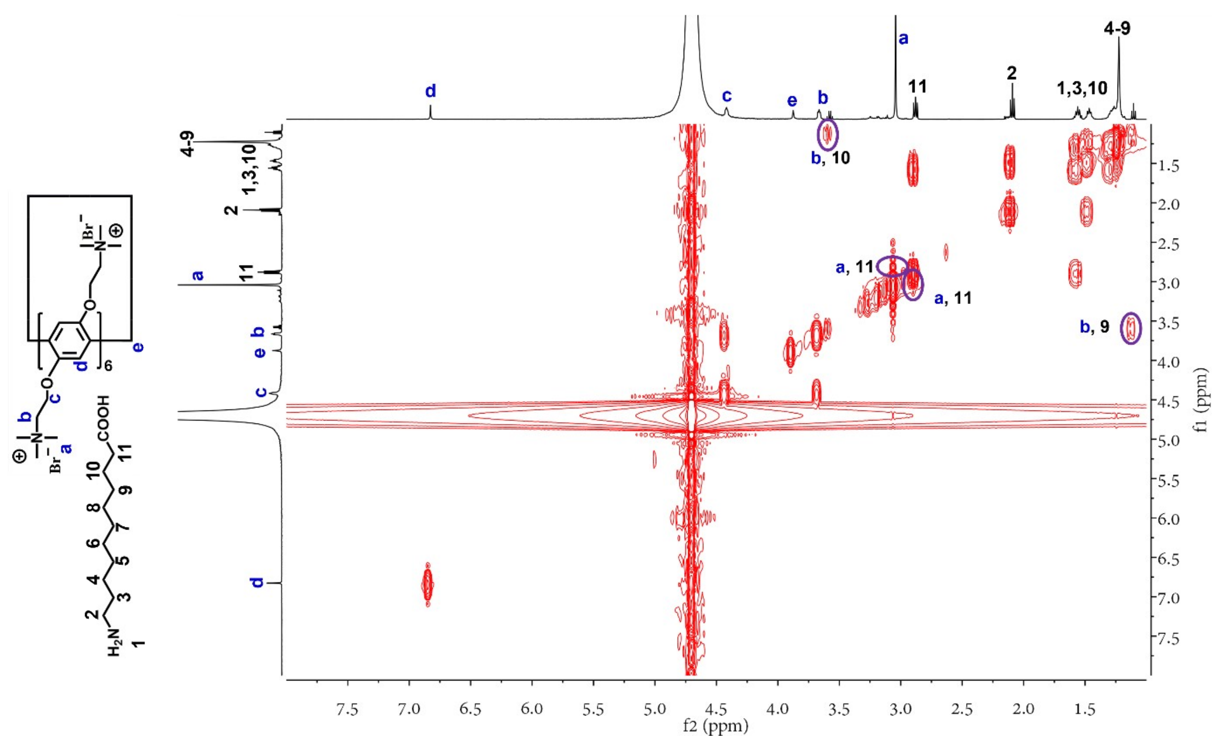

Fig. S3 Two-dimensional nuclear Overhauser spectroscopy (2D-NOESY) of CP6⊃11-AUA compounds.

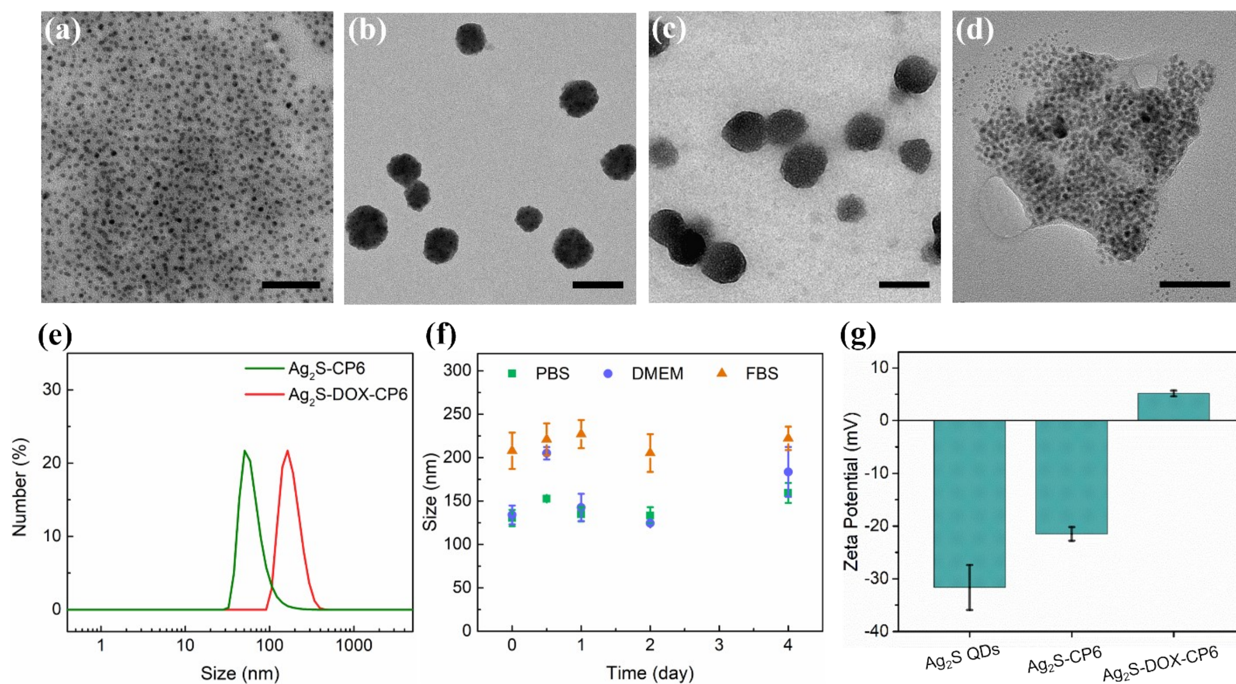

Fig. S4 Characterization of the co-delivery system. (a-d) TEM and HRTEM images of Ag_2S QDs (a), Ag_2S-CP6 (b) and Ag_2S-DOX-CP6 (c, d). Scale bars: 50 nm (a, b), 100 nm (c), 50 nm (d). (e) Size distributions of Ag_2S-CP6 and Ag_2S-DOX-CP6 measured by DLS.(f) The changes of size of Ag_2S-DOX-CP6 with the increase of time in PBS, DMEM and FBS. (g) Zeta potential of Ag_2S QDs, Ag_2S-CP6 and Ag_2S-DOX-CP6 measured by DLS.

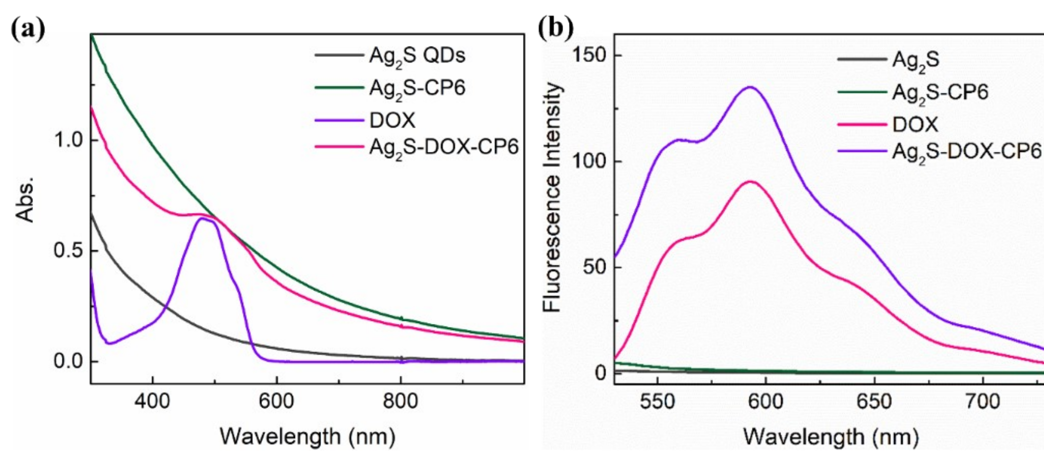

Fig. S5 UV-vis (a) and fluorescence spectra (b) of Ag_2S, Ag_2S-CP6, DOX and Ag_2S-DOX-CP6.

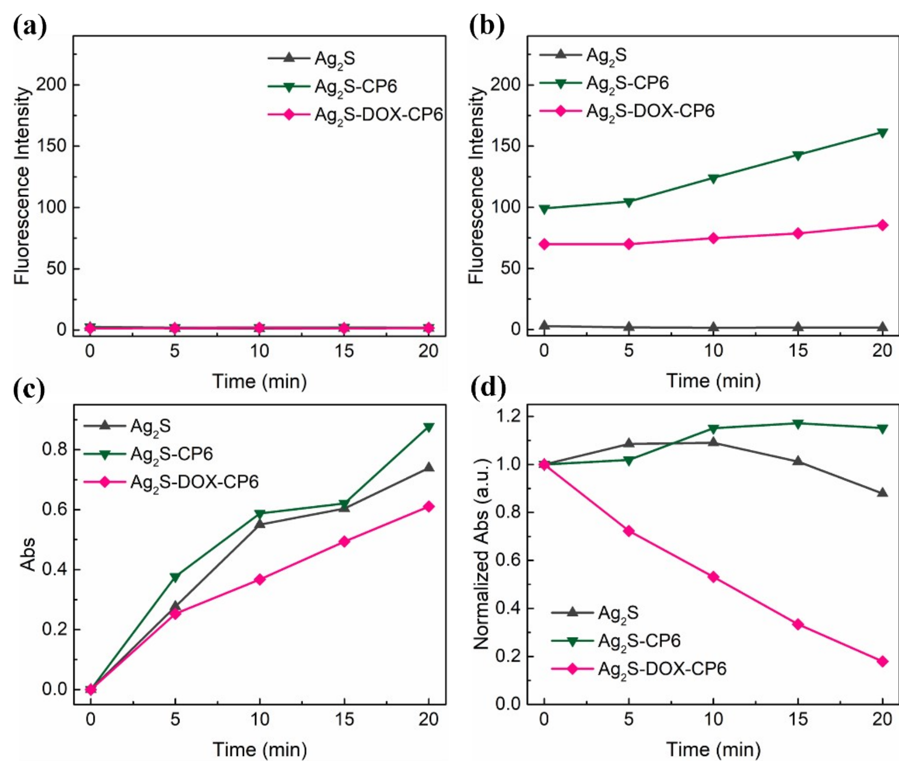

Fig. S6 (a) ·OH detection using CCA (808 nm, 2 W/cm^2) for Ag_2S, Ag_2S-CP6 and Ag_2S-DOX-CP6. (b) H_2O_2 detection using HPA (808 nm, 2 W/cm^2). (c) $\cdot O_2^-$ detection using NBT (808 nm, 2.0 W/cm^2). (d) $^1O_2$ detection using DPBF (808 nm, 2.0 W/cm^2).

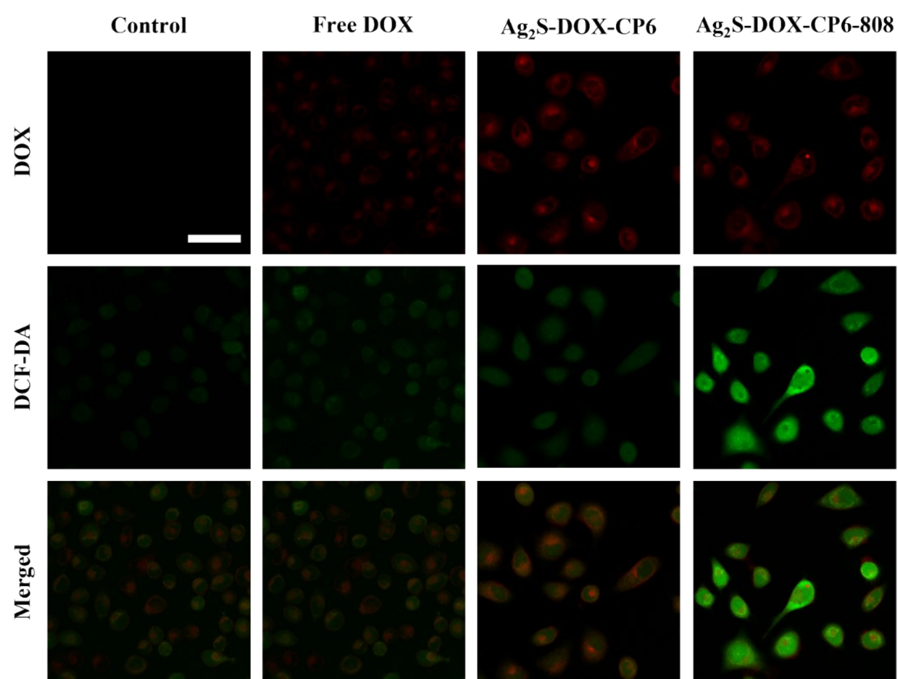

Fig. S7 CLSM images of intracellular ROS detection in MCF-7 cells incubated with free DOX and Ag_2S-DOX-CP6 (DOX concentration of 5 μg/mL) with or without irradiation (808 nm, 1.0 W/cm^2, 1 min). Scale bar: 50 μm.

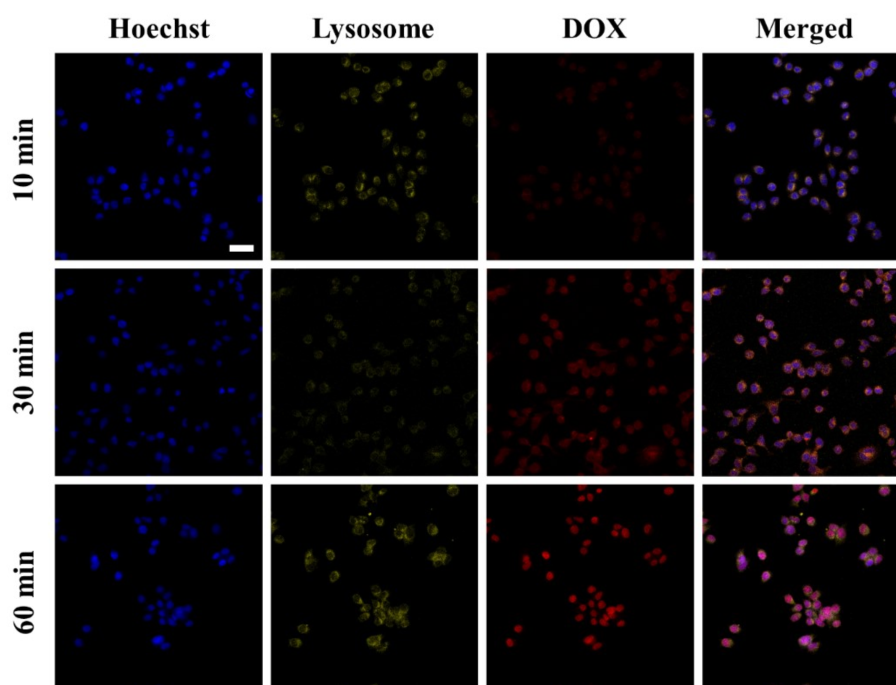

Fig. S8 CLSM images of MCF-7 cells incubated with free DOX (DOX concentration: 5 μg/mL) for 10, 30 and 60 min. The cells are treated with Hoechst 33342 (EM: 430-490 nm; EX: 405 nm) and LysoTracker Deep Red (EM 650-720 nm; EX 635 nm) to stain the nucleus and lysosome. DOX were excited at the wavelength of 488 nm, and the fluorescence images at emission wavelengths are at 550-640 nm. Scale bar: 50 μm.

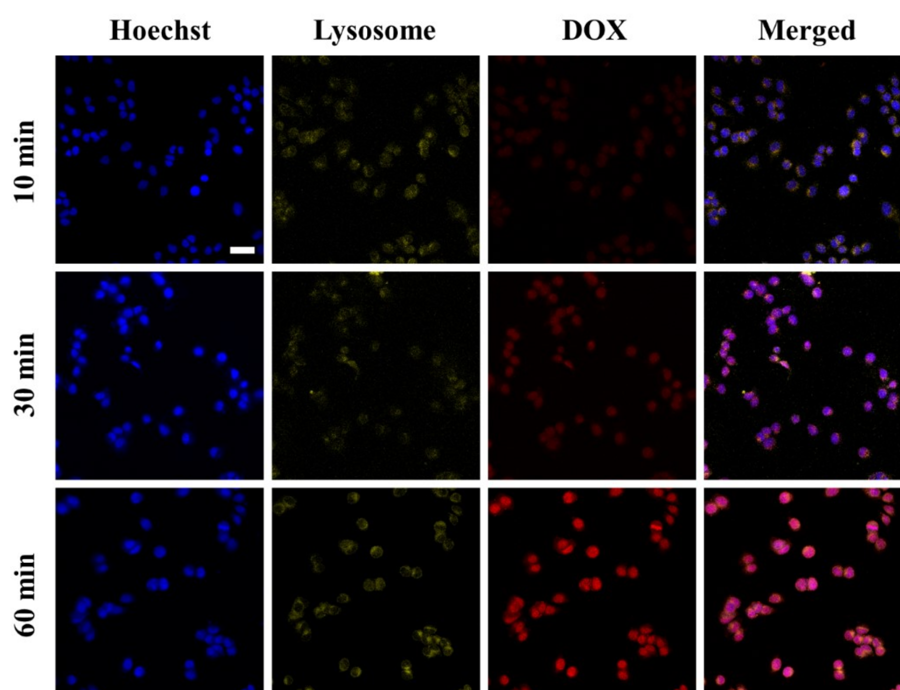

Fig. S9 CLSM images of MCF-7 cells incubated with Ag_2S-DOX-CP6 (DOX concentration: 5 μg/mL) for 10, 30 and 60 min. The cells are treated with Hoechst 33342 (EM: 430-490 nm; EX: 405 nm) and LysoTracker Deep Red (EM 650-720 nm; EX 635 nm) to stain the nucleus and lysosome. DOX were excited at the wavelength of 488 nm, and the fluorescence images at emission wavelengths are at 550-640 nm. Scale bar: 50 μm.

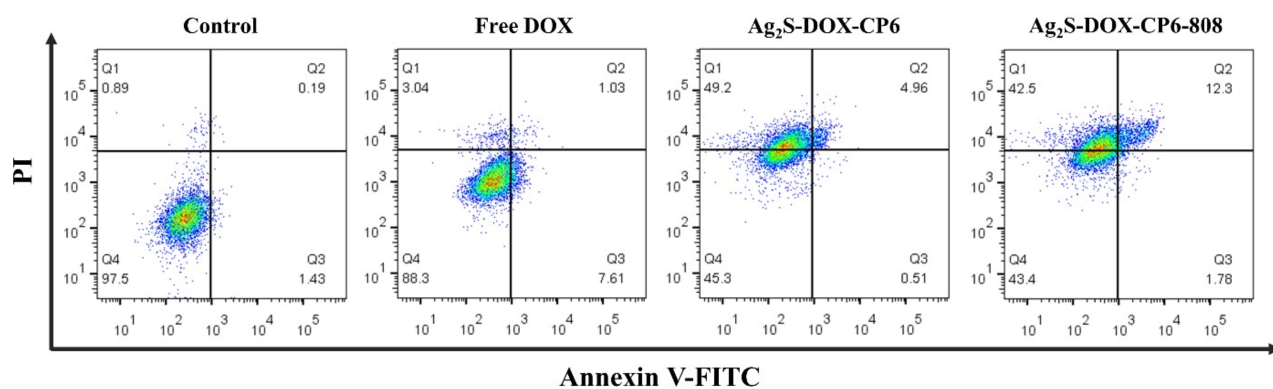

Fig. S10 Flow cytometry test for apoptosis and cell viability in the MCF-7 cells treated with free DOX, Ag_2S-DOX-CP6 and Ag_2S-DOX-CP6 under 808 nm irradiation (1.0 W/cm^2, 1 min) using Annexin V-FITC/propidium iodide (PI) kit. The DOX concentration are 2 μg/mL MCF-7 cells. n = 3.

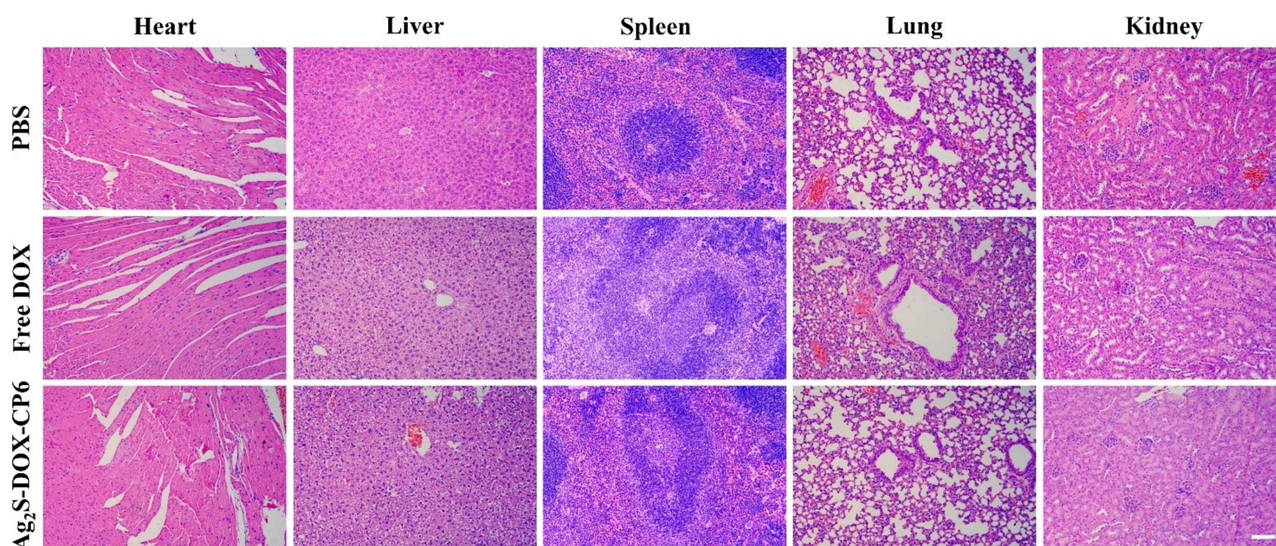

Fig. S11 H&E staining of major organs (heart, liver, spleen, lung and kidney) from male ICR mice after 15 days of intravenous injection of PBS, free DOX and Ag_2S-DOX-CP6. Scale bar: 100 μm.

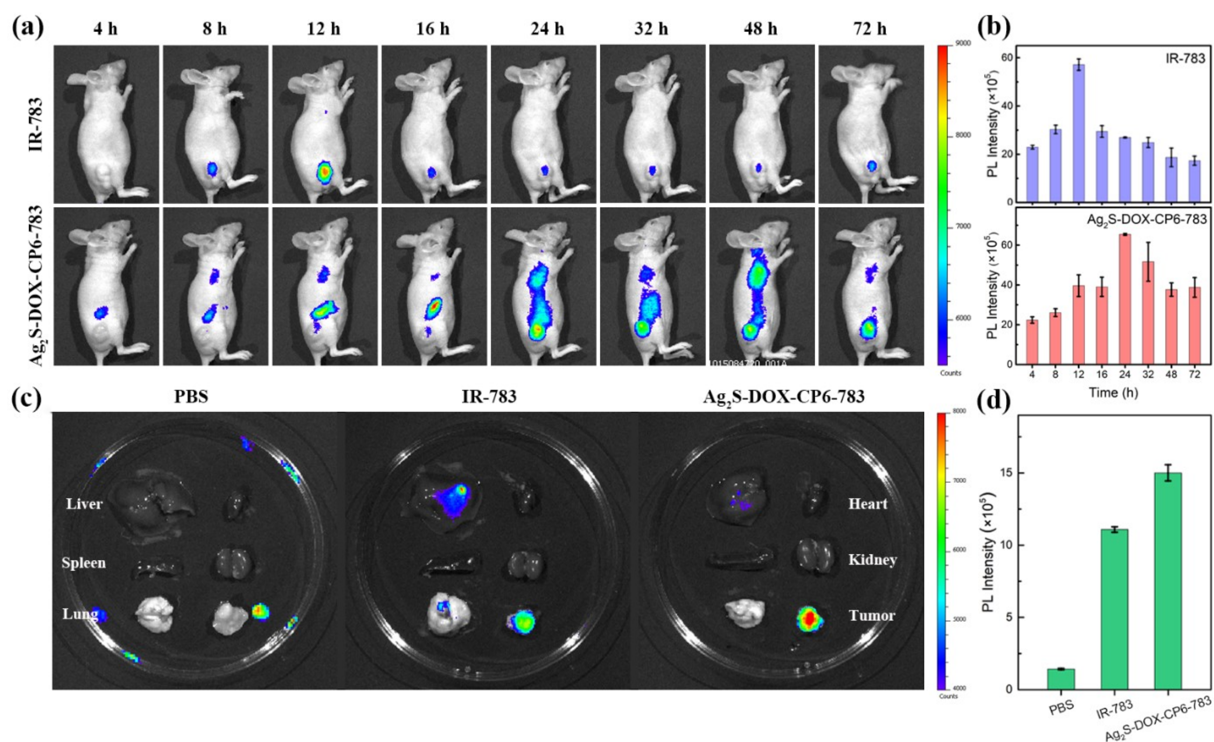

Fig. S12 (a, b) *In vivo* fluorescence distributions and relative fluorescence intensity of tumor at different time points after injection of free IR-783 probe and Ag_2S-DOX-CP6 labeled by IR-783 (Ag_2S-DOX-CP6-783). (c) *In vitro* fluorescence images of tumors and major organs of liver, spleen, lung, heart and kidney at 24 h postinjection of free IR-783 probe and Ag_2S-DOX-CP6-783. (d) Tumor fluorescence intensity from mice after 24 h injection of free IR-783 probe and Ag_2S-DOX-CP6-783.

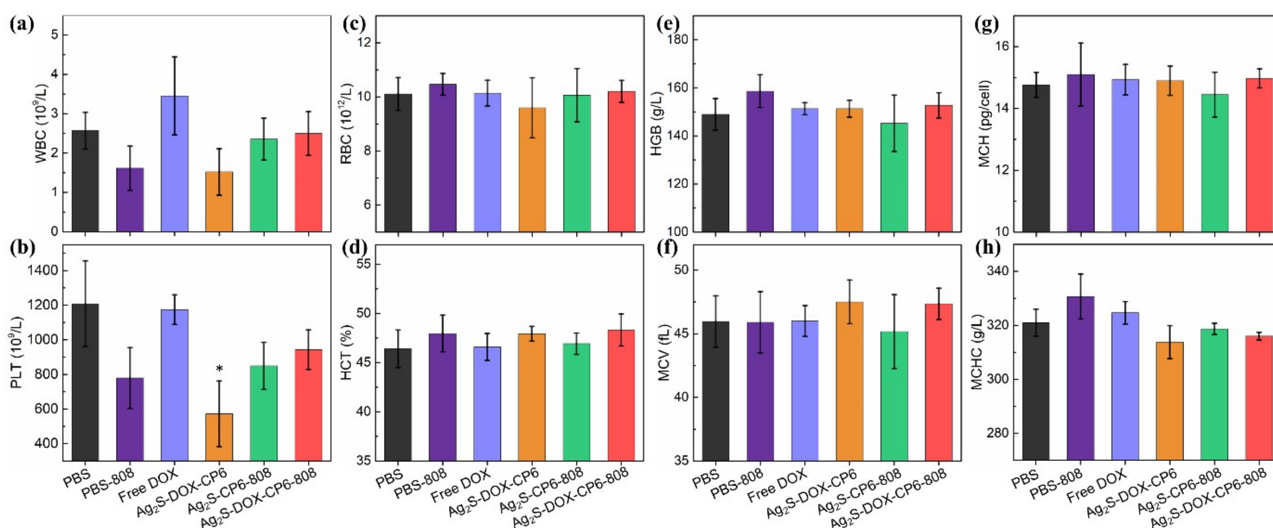

Fig. S13 Hematology results from mice treated with intraperitoneal injection of PBS, PBS-808, free DOX, Ag_2S-DOX-CP6, Ag_2S-CP6-808, Ag_2S-DOX-CP6-808. These results show mean and standard deviations of (a) white blood cells (WBC), (b) platelet (PLT), (c) red blood cell (RBC), (d) hematocrit (HCT), (e) hemoglobin (HGB), (f) mean corpuscular volume (MCV), (g) mean corpuscular hemoglobin (MCH); (h) mean corpuscular hemoglobin concentration (MCHC). Bars represent mean ± standard deviation (n = 3). *Represents a significant difference from the control group (*P<0.05).

S3. References

S1. L. Feng, F. He, B. Liu, G. Yang, S. Gai, P. Yang, C. Li, Y. Dai, R. Lv and J. Lin, *Chem. Mater.*, 2016, **28**, 7935-7946.