Electronic Supplementary Material (ESI) for Nanoscale Horizons.

Supporting Information

Macrophage-hitchhiked supramolecular aggregates of CuS nanoparticles for enhanced tumor deposition and photothermal therapy

Junyan Li,[+,a] Qian Cheng,[+,a] Ludan Yue,[+,a] Cheng Gao,[a] Jianwen Wei,[a] Yuanfu Ding,[a] Yitao Wang,[a] Ying Zheng,[a,b] and Ruibing Wang[*,a,b]

[a] State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Taipa, Macau, China.

[b] MOE Frontiers Science Center for Precision Oncology, University of Macau, Taipa, Macau, China.

*rwang@um.edu.mo

Table of Content

Experimental section

Materials and Equipment

Copric chlorides, Sodium citrate and 6-Ferrocenylhexanethiol (SH-Fc) were purchased from Aladdin. Sodium sulfide was purchased from Shanghai Machlin Biochemical Co., Ltd (Shanghai, China). Per-6-thio-β-cyclodextrin (SH-β CD) was purchased from Zhiyuan Biotechnology Co., Ltd (Shandong, China), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was supplied by Amresco. 2',7'-dichlorofluorescin diacetate (DCHFDA) and 4',6-diamidino-2-phenylindole (DAPI) were purchased from Invitrogen (USA). Hoechst 33342, Lyso-tracker Red, NO Detection Kit and Annexin/V-FITC Detection Kit were purchased from Beyotime Biotechnology Co., Ltd (Shanghai, China). Dulbecco's modified Eagle medium and fetal bovine serum were purchased from Gibco (USA). All other reagents were obtained from commercial sources and used without further purification. Dialysis was performed using a Slide-A-Lyzer dialysis cassette (MWCO, 5 kDa), Milli-Q water was purified with a Milli-Q Integral system from Merck Millipore.

The size and zeta potential of NPs were determined by dynamic light scattering (DLS) at 25 °C with a Zetasizer (Malvern. Co., UK). Transmission electron microscopy (TEM) images were obtained using a Tecnai G20 TEM (FEI, Co., USA). The ^{1}H NMR spectra were performed on a Bruker Ultra Shield 600 PLUS NMR spectrometer. A FACS flow cytometer (Beckman coulter) was used to analyze cell apoptosis and cell viability was measured by a multimode microplate reader (FlexStation 3). Intracellular aggregations and ROS production were observed with a confocal laser scanning microscopy (CLSM, Zeiss LSM710). An inverted fluorescent microscope (Olympus IX73) was employed to observe the migration of macrophages. Copper content was measured by inductively coupled plasma mass spectrometry (ICP-MS). In vivo images of CuS NPs were obtained with a computed tomography (CT) system (SuperArgus, Sedecal).

Raw 264.7 and B 16 cells were cultured in 25 cm^2 flasks using Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS, 100 $U \cdot mL^{-1}$ penicillin and 100 $mg \cdot mL^{-1}$ streptomycin. All the cells were finally cultured in an incubator (37 °C; 5% CO_2). B 16 cells were sub-cultured regularly using trypsin/EDTA. All cell lines were obtained from ATCC (Manassas, VA, USA).

Synthesis and characterization

$CuCl_2$ (16.9 mg, 0.1 mmol) and Na_3Cit (28.0 mg, 0.02 mmol) were dissolved into 100 mL Milli-Q water, 1 mL Na_2S (1 mM) was added to this solution and mixed for 5 min. The reaction mixture was heated to 90°C for 15 min until a dark green solution was observed.[1] CuS NPs were obtained after centrifugation (10000 rpm; 10min). Followingly, SH-β CD (11.51 mg, 10 μmol) and SH-Fc (1.511 mg, 5 μmol) were dissolved in Milli-Q water and tetrahydrofuran, respectively. Then solution of SH-β CD were added into CuS NPs (100 μg/mL; 100mL) and stirred overnight. β-cyclodextrin capped CuS NPs (CD-CuS) were obtained after purifying with dialysis bag (MWCO, 5 kDa) for 3 days to remove the unreacted agents. Ferrocene capped CuS NPs (Fc-CuS) were obtained with a similar procedure. The obtained NPs were stored at 4 °C.

Fc-CuS (containing 100 μg CuS) NPs were soaked in 1 mL aqua regia for measurement of Fe content via ICP-MS. Fe concentration of this solution was 0.05711 μmol/mL, indicating there was 0.05711 μmol Fc connected onto surface of 100 μg CuS and the final concentration of Fc was 0.5711 μmol/mg CuS. The amount of CD was evaluated via a similar method, Excessive Fc dissolved in tetrahydrofuran was added into CD-CuS aqueous solution firstly to from the 1:1 host-guest complexes with CD, and dialysis bag was used to remove free Fc. After digestion by aqua regia, the amount of Fc was evaluated via ICP-MS. There was 0.3671 μmol Fc per milligrams CuS, which indicated that the concentration of CD was ca. 0.3671 μmol/mg CuS.

Self-assembly and ROS-induced disassembly

Aqueous suspensions of CD-CuS and Fc-CuS NPs at the same concentration (100 μg/mL) were mixed to get CuS aggregations (Agg). DLS size of Agg was observed with a Zetasizer. The sample was dropped on a copper mesh and dried under vacuum for morphology observation by TEM. For ROS-induced disassembly, Agg (1 mL) was incubated with H_2O_2 at concentration of 1 mM. The DLS size was measured 2, 4, 8, 12, 24 hours later.

Stability in different pH values

As professional phagocytes, macrophages have high phagocytic capacity towards extracellular materials whose process may involve in acidic lysosomes.[2, 3] To verify the stability of Agg in different pH values, Agg was

incubated in aqueous suspension when pH = 7.4; 6.8; 6.0 and 5.0. DLS size was measured with a Zetasizer at 12 h, 24 h, 48 h, 72 h. The almost unchanged DLS size and morphology indicated the stability of aggregates.

Photothermal potency of nanoparticles

An aqueous suspension of nanoparticles (1 mL) at different concentrations (12.5, 25, 50, 100, 200 μg/mL) was added into a quartz cuvette and irradiated with an 808 nm laser (1W/cm^2; 5min). The increased temperature was recorded per 30 s using a digital thermometer. The aqueous suspension of nanoparticles was cooled to room temperature before next 5-min lasering until 5 cycles to demonstrate the photothermal stability.

According to Roper's report[4], the photothermal conversion efficiency was caculated from Equation (1) and the energy balance can be expressed as Equation (2):

$$\eta = \frac{hA(T_{max}-T_{surr})-Q_{dis}}{I(1-10^{-A808})} \quad \text{.....(1)}$$

$$\sum_i m_i C_{p,i} \frac{dT}{dt} = \sum_j Q_j \text{.....(2)}$$

Where h is heat transfer coefficient, S is the surface area of the container, Q_{dis} mans heat absorbed by the container, η is the photothermal conversion efficiency, T_{max} represents the maximum temperature during irradiation, T_{surr} represents surrounding environment temperature, I is the laser power, and A808 is the absorbance of the sample at 808 nm, m and C_p are the mass and heat capacity, respectively. Q_{dis} was calculated indepently to be 0.516 J/s. In order to get only unkown value the hA, θ and τ_s are introduced in Equation (3),(4):

$$\theta = \frac{T-T_{surr}}{T_{max}-T_{surr}} \quad \text{.....(3)}$$

$$\tau_s = \frac{\sum_i m_i C_{p,i}}{hA} \quad \text{.....(4)}$$

According to Roper's report[4], $In\ \theta = -\frac{t}{\tau_s}$, the time constant for heat transfer from the system is determined to be τ_s = 196 s by applying linear time data versus ln θ from the cooling stage. And hA is deduced to be 0.0214 W/℃ according to Equation (4). By substituting hA into Equation (1), the photothermal conversion efficiency of CuS NPs and aggregates was calculated as 28.51% and 28.43%, respectively.

Biocompatibility and apoptosis assay

Cytotoxicity of nanoparticles was evaluated by MTT assays for both macrophages and B 16 cells. Typically, macrophages and B 16 cells were respectively seeded in a 96-well plate at a density of 1 × 10^4 per well containing 100 μL DMEM, and incubated in a humidified atmosphere with 5 % CO_2 at 37 °C for 24 h. Then culture medium was replaced with 100 μL fresh DMEM containing nanoparticles at different concentrations (6.25, 12.5, 25, 50, 100, 200 μg/mL) for 24 h, while B 16 cells incubated with NPs received irradiation at 12 h and were incubated for another 12 h. After discarding the culture medium, 100 μL fresh medium containing MTT solution (5 mg/mL, 10 μL) was added into each well and incubated for an additional 4 h. After disposal of supematant, 100 mL DMSO was added and shaken to dissolve the formazan. Finally, the optical density (OD) values at 490 nm of each well was measured by a multiwell plate reader. The relative cell viability was calculated as follows:

$$\text{Relative cell viability} = (OD_{sample} —— OD_{blank}) / (OD_{control} —— OD_{blank}) \times 100\ \%$$

For apoptosis assays, B 16 cells were seeded into 12-well plates and incubated for 24 h (37 °C; 5% CO_2). Then CuS NPs, CD-CuS, Fc-CuS and Agg were added at a concentration of 100 μg/mL. 12 hours later, B 16 cells received irradiation (1W/cm^2; 5 min) and were incubated for another 12 hours. Finally, cells were harvested from 12-well plates and washed with fresh phosphate-buffered saline (PBS). Cells finally sorted and analyzed by a flow cytometer after incubation with Annexin/V-FITC Detection Kit.

Co-localization of CD-CuS and Fc-CuS in macrophage by confocal laser scanning microscopy (CLSM)

Firstly, Cy 5.5 and FITC labelled CD-CuS and Fc-CuS were synthesized: SH-PEG 2000-Cy 5.5 (2 mM, 200 μL) was added into aqueous suspension of CD-CuS (100 μg/mL, 10 mL) and stirred overnight. Cy 5.5 labelled CD-

CuS NPs (CD-CuS-Cy 5.5) were obtained after purifying with a dialysis bag (MWCO, 5 kDa) for 3 days. FITC labelled Fc-CuS NPs were obtained similarly. Then macrophages were seeded in confocal dishes and incubated with CD-CuS-Cy 5.5 for 4 hours. After disposal of supematant, Fc-CuS-FITC was added and incubated for another 4 hours. The culture medium was discarded and cells were washed with fresh PBS before confocal imaging. The Mander's Colocalization Coefficient (MCC) was used to quantify the colocalization of NPs in macrophages, which was simply calculated from the proportion of signals from one channel overlaping with signals from the other channel.

Intracellular copper content detection

Firstly, macrophages were seeded in 6-well plate and incubated with CD-CuS for 4 hours. After disposal of supematant, Fc-CuS was added and incubated for another 4 hours. The culture medium was discarded and then cells were gently washed with fresh PBS. Finally, macrophages were removed with 0.5 mL fresh PBS and digested with aqua regia. Intracellular copper content was measured by ICP-MS.

For efflux experiments, CuS NPs internalized Macrophage (CuS-M) and Supramolecular Aggregates of NPs internalized Macrophage (Agg-M) were prepared after incubation with CuS alone, and CD-CuS and Fc-CuS sequentially, respectively. The CuS-M and Agg-M were incubated in fresh medium (the media contained 100 ng/mL LPS for Agg-M + LPS group) and removed and digested at 0, 1, 2, 4, 6, 12 and 24 h. The intracellular copper content was evaluated by ICP-MS and efflux percent was calculated as follows:

$$\text{Efflux percent} = \left(1-\frac{Intracellular\ copper\ content}{Intracellular\ copper\ content\ at\ 0h}\right) \times 100\ \%$$

Intracellular ROS and NO detection

Lipopolysaccharides (LPS) is a widely used stimulant for classically activated macrophages with the ability of producing ROS and NO.[5, 6] DCHFDA was used as a fluorescence singlet oxygen sensor through reacting with O_2 and resulting in a dramatic increase in fluorescence intensity.[7] Macrophages were seeded into confocal dishes or 96-well plates and then incubated with LPS for different hours (0, 1, 2, 4, 6) or at different concentrations (0 ng/mL, 25 ng/mL, 50 ng/mL, 100 ng/mL, 200 ng/mL, 400 ng/mL). After removing supematant, cells were respectively incubated with Hoechst 33342 and DCHFDA for 15 min. All treated cells were washed with PBS for CLSM images. For semiquantitative analysis of ROS production, activated macrophages were treated with DCFHDA for fluorescence intensity measurement (Excitation: 488 nm, Emission: 530 nm). Similarly, NO production by activated macrophages was measured through NO detection kit.

Cell migration analysis

Firstly, B 16 cells (1×10^5 cells) were seeded onto bottom wells in 600 μL DMEM medium, macrophages, CuS NPs internalized Macrophage (CuS-M) and Supramolecular Aggregates of NPs internalized Macrophage (Agg-M) were seeded in the top transwell chamber in 100 μL FBS-free DMEM medium. Blank DMEM served as control. The transwell inserts were collected 4 hours later and fixed with 4 % formaldehyde for 30 min. The upper side of transwell inserts were wiped with cotton swabs and stained with crystal violet for 15 min. Finally, the migration cells were observed by fluorescence microscope.

Biosafety assessment

C 57BL/6 female mice aging 6 weeks were divided into 5 groups (n=3) and intravenously injected with CuS NPs, CuS NPs internalized Macrophage (CuS-M), Supramolecular Aggregates of CuS NPs (Agg) and Supramolecular Aggregates of NPs internalized Macrophage (Agg-M) or PBS (control group). Blood was collected from the mice 24 h post injection, followed by centrifugation at 3000 rpm for 10 min. The supernant plasma fraction was stored at -80 °C for biochemistry analysis such as for alanine aminotransferase (ALT), aspartate aminotransferase (AST), Creatine Kinase (CK), Lactic Dehydrogenase (LDH), gamma-glutamyl transferase (γ-GT), creatinine, and urea. In addition, whole blood was collected for hematology parameters detection. such as white blood cell count, red blood cell count, hemoglobin, hematocrit, and platelets were measured. To investigate the tissue pathology, mice were anaesthetized, sacrificed, and major organs (heart, liver, spleen, lung and kidneys) were harvested for H&E staining.

Blood circulation behavior

For blood circulation analysis, healthy female BALB/c mice (n = 3) were intravenously injected with 100 μL of CuS, Agg, CuS-M and Agg-M solution via the tail-vein. Blood was drawn from the suborbital space at 2, 4, 6, 12, and 24 h post injection, wet-weighed, and dissolved in aqua regia (HCl: HNO3 of 3:1 by volume). The concentration of nanoparticles was quantified with the concentration of copper determined by ICP-MS.

Biodistribution

C 57BL/6 female mice aging 6 weeks was injected with B 16 cells at destiny of 1×10^6 per 100 μL PBS on the left flank to obtain melanoma-bearing mice. CuS, Agg, CuS-M, Agg-M were intravenously injected to mice (Concentration of CuS: 2 mg/mL) after tumor volume reached about 100 mm^3. Then mice were anesthetized for CT imaging at 0 h, 2 h, 4 h, 6 h, 12 h and 24 h. 24 h post-injection, all mice were sacrificed and tumors and organs (heart, liver, spleen, kidney and lung) were collected for CT ex-vivo imaging. Besides, tumors and organs were crushed and soaked in aqua regia to dissolve copper for quantitative analysis by ICP-MS.

Moreover, DiI (dye staining cell membrane) stained Agg-M and CuS-M (CuS NPs were labelled with Cy 5.5) were utilized for indicating the location of macrophage (green channel) and NPs (red channel) in the tissues collected after administration for 2 h.

In vivo PT tumor imaging

C 57BL/6 female mice aging 6 weeks was injected with B 16 cells at destiny of 1×10^6 per 100 μL PBS on the left flank to obtain melanoma-bearing mice. PBS, CuS, Agg, CuS-M, Agg-M were intravenously injected to mice (Concentration of CuS: 2 mg/mL), 4 h post-injection, all mice were anesthetized for irradiation on tumor sites (808 nm; 1 W/cm^2). Temperature increase was monitored by $TiS60^+$ THRMAL IMAGER.

In vivo anti-tumor study

Melanoma tumor-bearing mice were randomly separated into 5 groups (n = 5 in each group) after tumor volume reached about 100 mm^3. CuS NPs, CuS-M, Agg and Agg-M were i.v. injected into the tail vein of mice, respectively, every 3 days during 15-day-treatment (CuS concentration: 2mg/mL, 100μL). For the control group, 100 μL PBS was injected intravenously. 4 hours post-injection, all mice were anesthetized and tumor regions were exposed to an 808 nm NIR laser for 5 min at the destiny of 1W/cm^2. Tumor volume and body weight were measured daily during treatment. Finally, all mice were sacrificed to obtain tumors and organs for hematoxylin and eosin (H&E) staining and terminal deoxynucleotidyl transferased dUTP nick end labeling (TUNEL) staining.

Besides, fluorescent imaing of the tumor tissues collected from mice treated with CuS-M and Agg-M (Carrier macrophages stained by DiI, all macrophages were immunostained with F4/80) was used to quantify "carrier" macrophage accumulated in the tumor, in comparison to all macrophages (F4/80+).

Result and discussion

All experiments were performed at least three times and results are given as mean ± standard deviation (n=3).

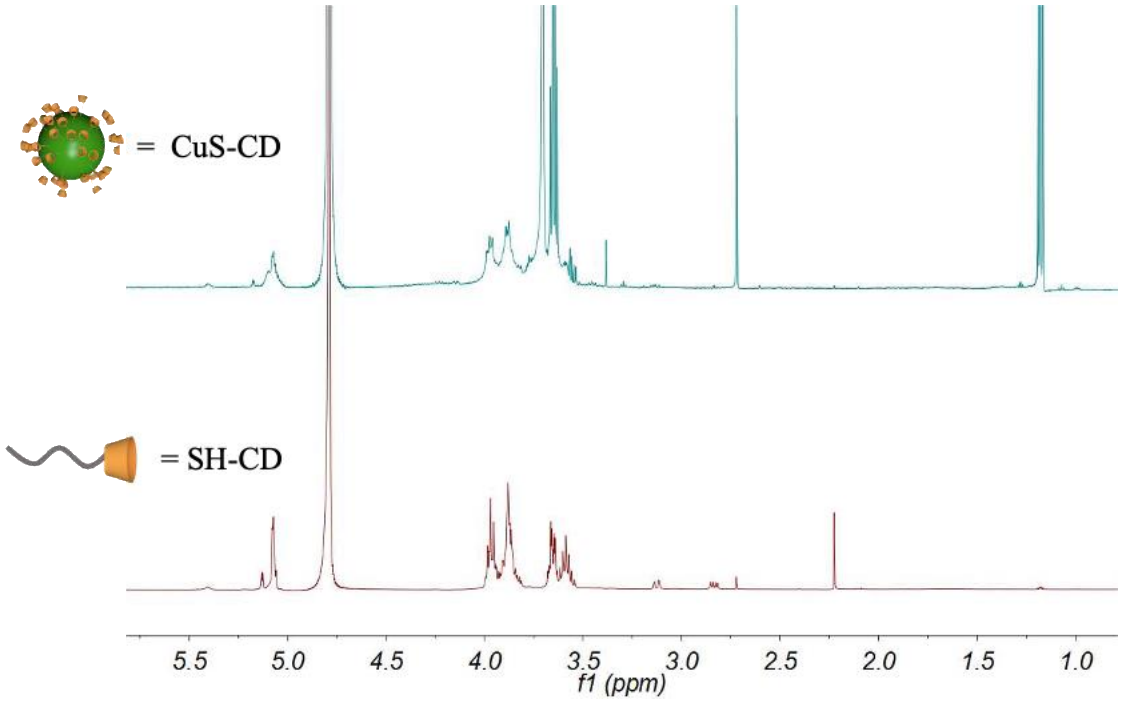


Figure S1. ^{1}H NMR spectra of SH-CD and CD-CuS.

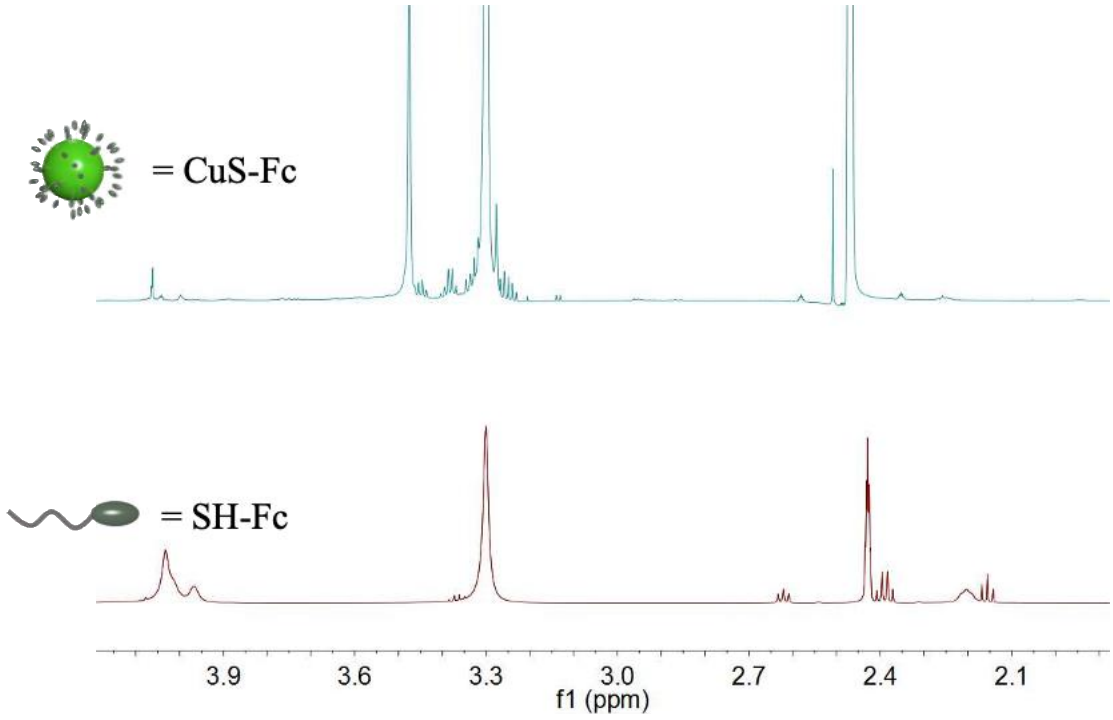


Figure S2. ^{1}H NMR spectra of SH-Fc and Fc-CuS.

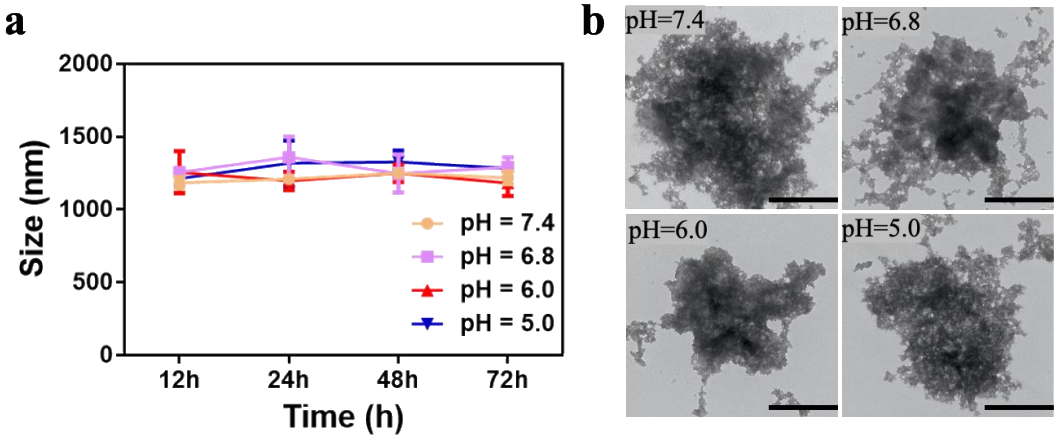


Figure S3. a) DLS size changes of CuS aggregations at different pH; b) TEM images of CuS aggregations at different pH. Scale bar: 500 nm.

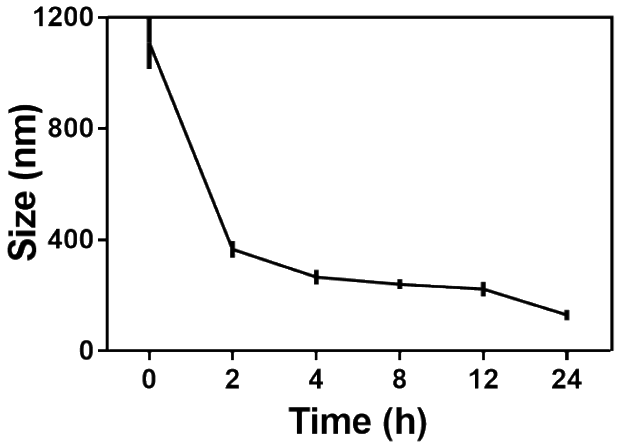


Figure S4. DLS size changes of CuS aggregations incubated with 1 mM H_2O_2.

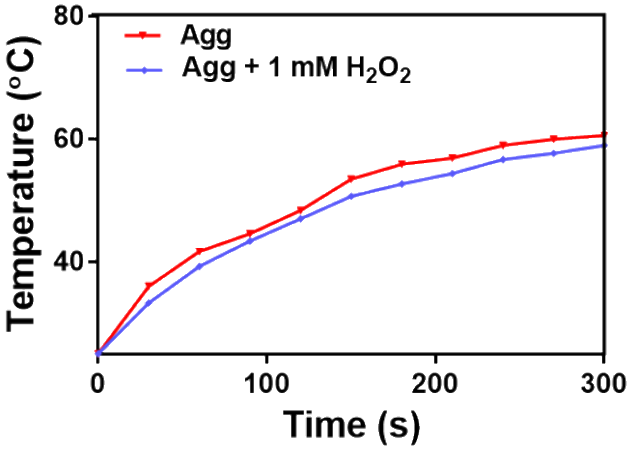


Figure S5. Temperature changes of CuS aggregations (Agg) and CuS aggregations (Agg) + 1 mM H_2O_2 irradiated with an 808 laser (1W/cm^2; 5 min).

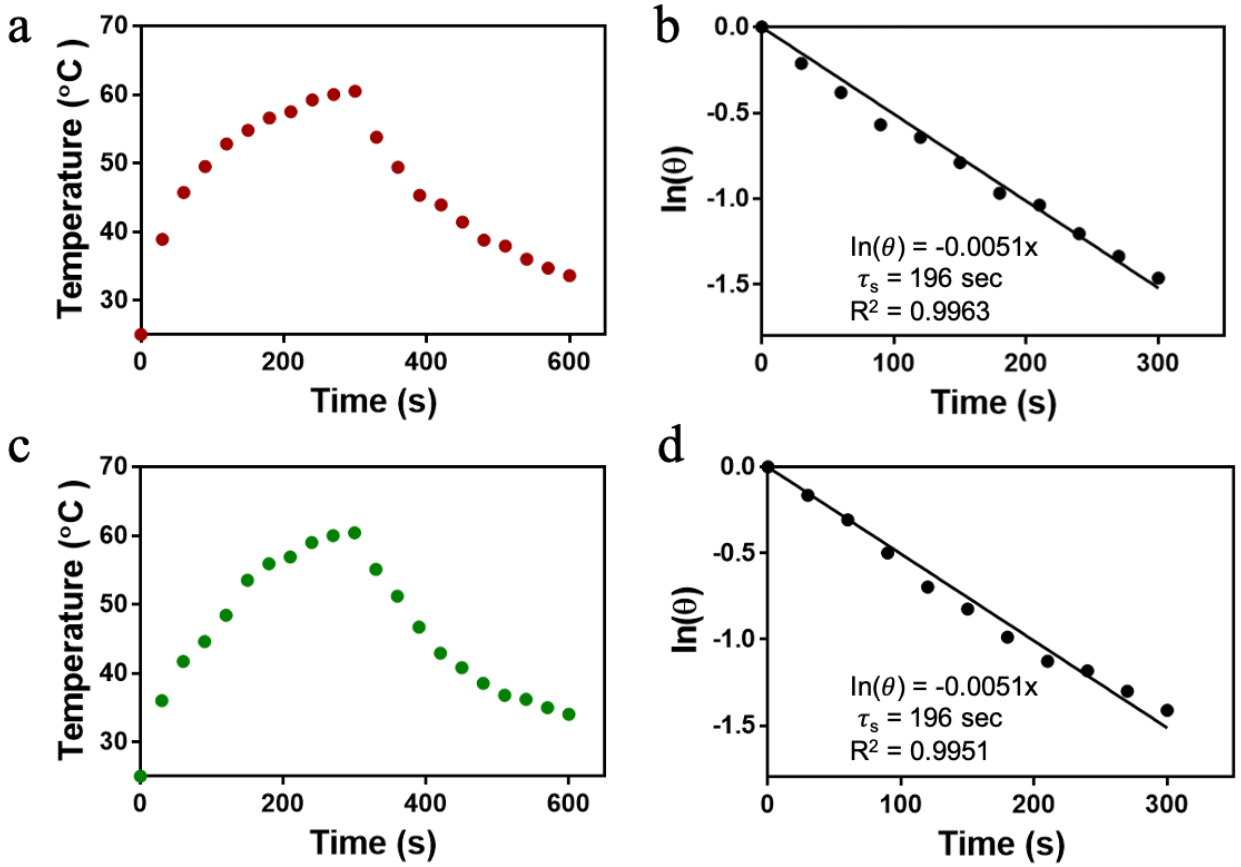


Figure S6. Temperature changes of (a) CuS NPs and (c) CuS aggregations irradiated with an 808 laser (1W/cm^2), in which the irradiation lasted for 5 min, and then the laser was shut off; (b, d)Time constant for heat transfer from the system is determined to be τ_s = 196 s by applying linear time data versus ln θ from the cooling stage.

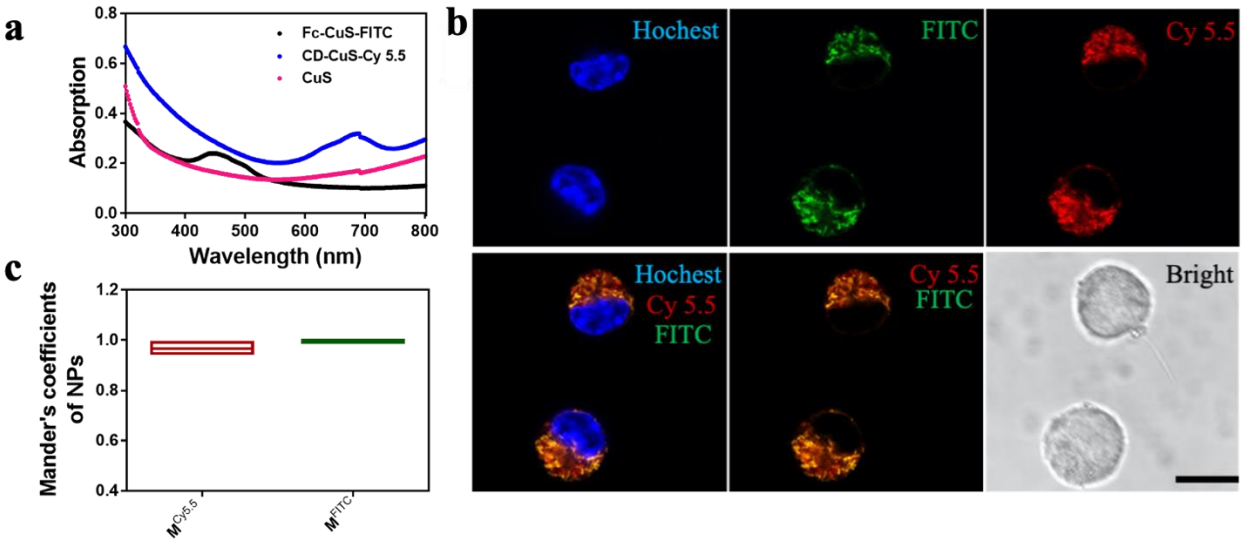


Figure S7. Co-localization of CD-CuS and Fc-CuS in macrophages: a) UV-vis-NIR absorption spectra of CuS, FITC labelled Fc-CuS(Fc-CuS-FITC) and Cy 5.5 labelled CD-CuS(CD-CuS-Cy 5.5); b) CLSM images of aggregates internalized macrophages stained with Hoechst 33342; c) Mander's colocalization coefficient of CD-CuS-Cy 5.5 with Fc-CuS-FITC. All data were presented as mean ± s.d.(n=3). $M^{Cy5.5}$: Proportion of overlapped Cy5.5 fluorescence; M^{FITC}: Proportion of overlapped FITC fluorescence.

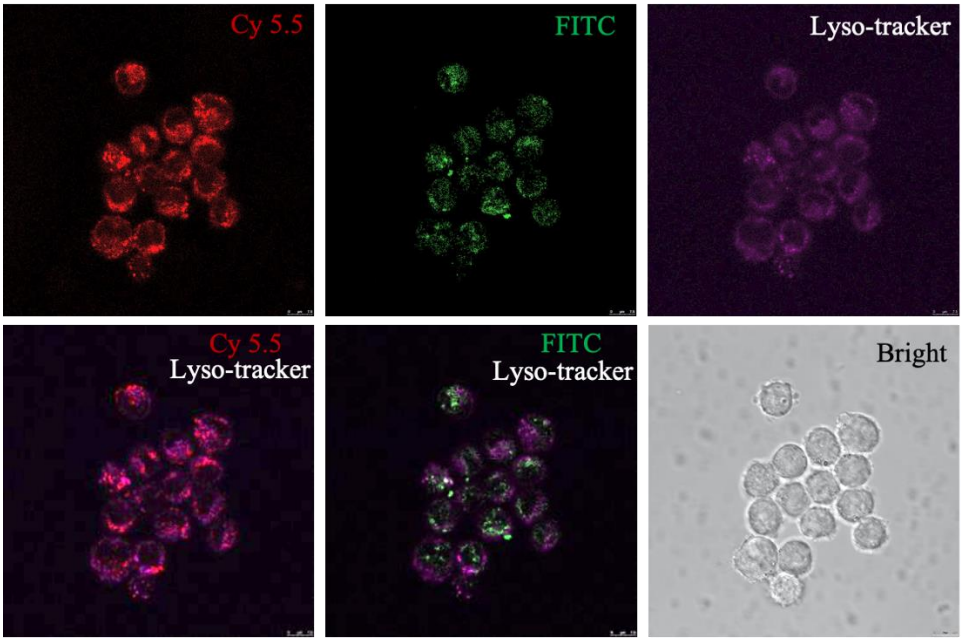
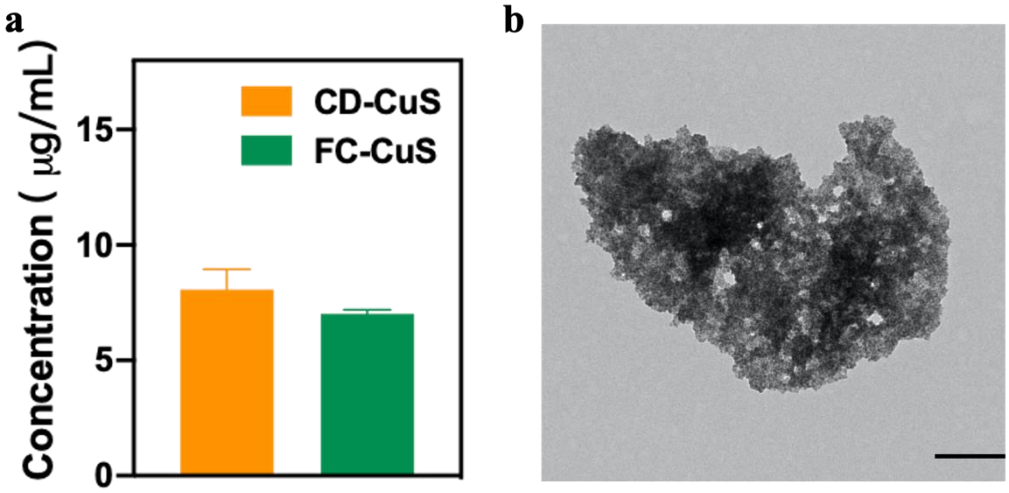


Figure S8. Localization of NPs (labeled with Cy5.5 and FITC, respectively) and lysosome (stained with Lyso-tracker Red) in macrophages: CLSM images of macrophages with intracellular CuS aggregates.



FigureS9. a) Concentration of intracellular CD-CuS and Fc-CuS. b) TEM images of the mixture of CD-CuS and Fc-CuS at proportion of 1:0.8. Scale bar: 200 nm.

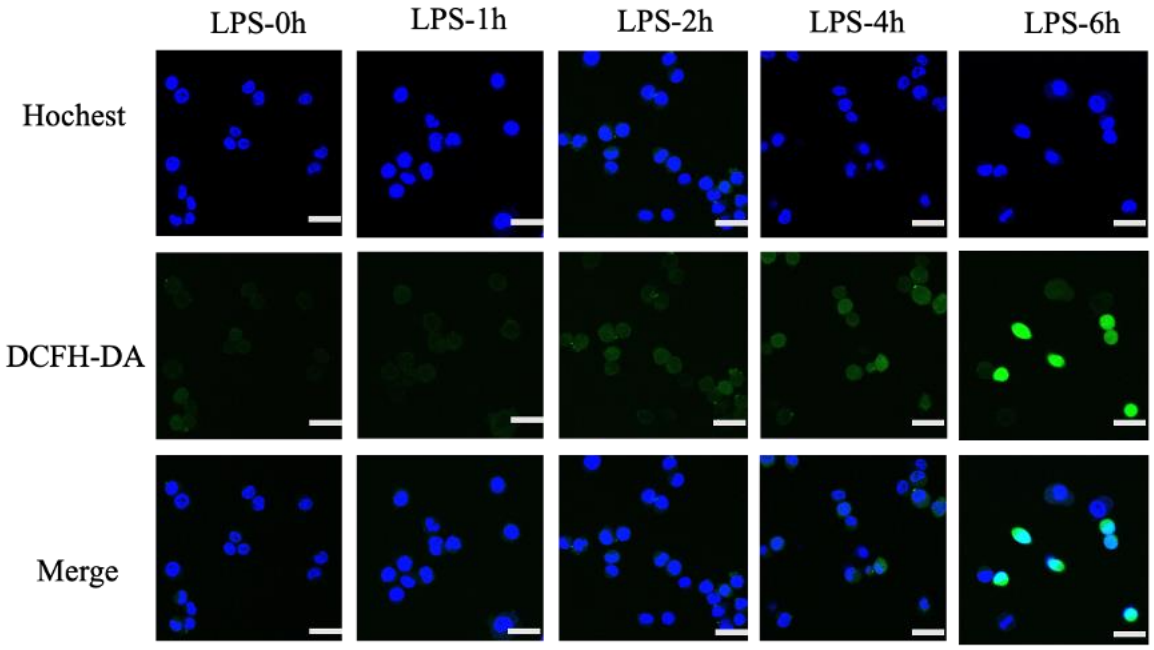


Figure S10. CLSM images of intracellular ROS in LPS-activated macrophages. Macrophages were incubated with 100 ng/mL LPS for different durations. The nucleus was stained by Hoechst 33342. Scale bar: 25 μm.

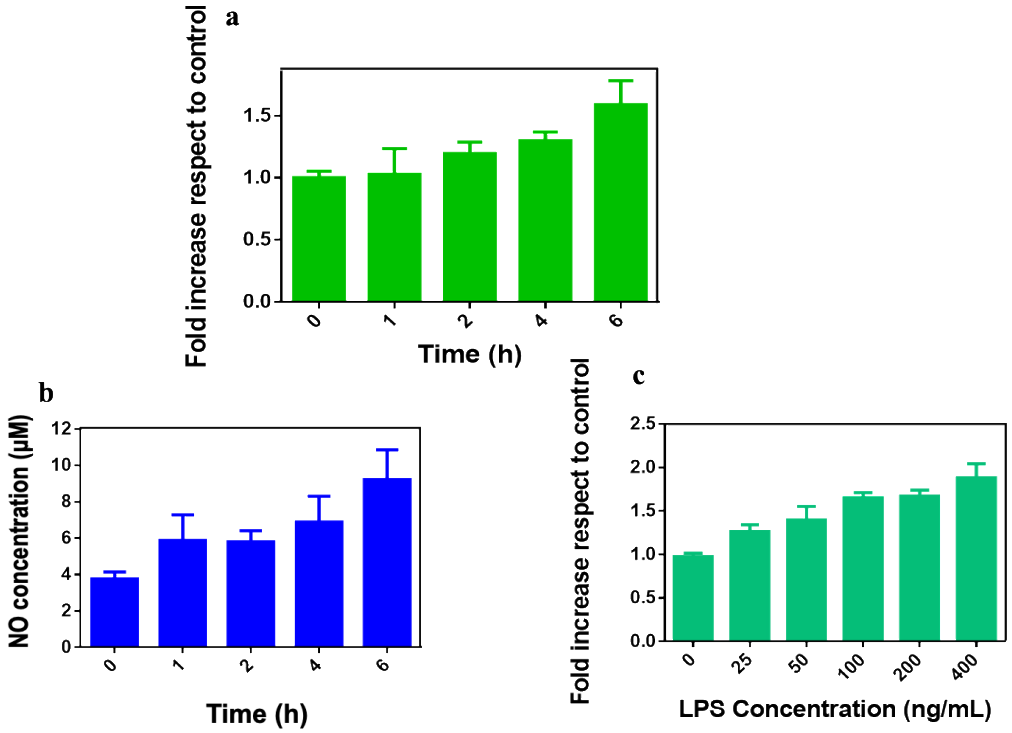


Figure S11. Analysis of Intracellular ROS and NO production in LPS-activated macrophage: a) Semiquantitative analysis of ROS production in macrophages incubated with LPS (100 ng/ml); b) NO production of macrophages incubated with LPS (100 ng/ml); c) Semiquantitative analysis of ROS production in macrophages incubated with different concentrations of LPS (6h).

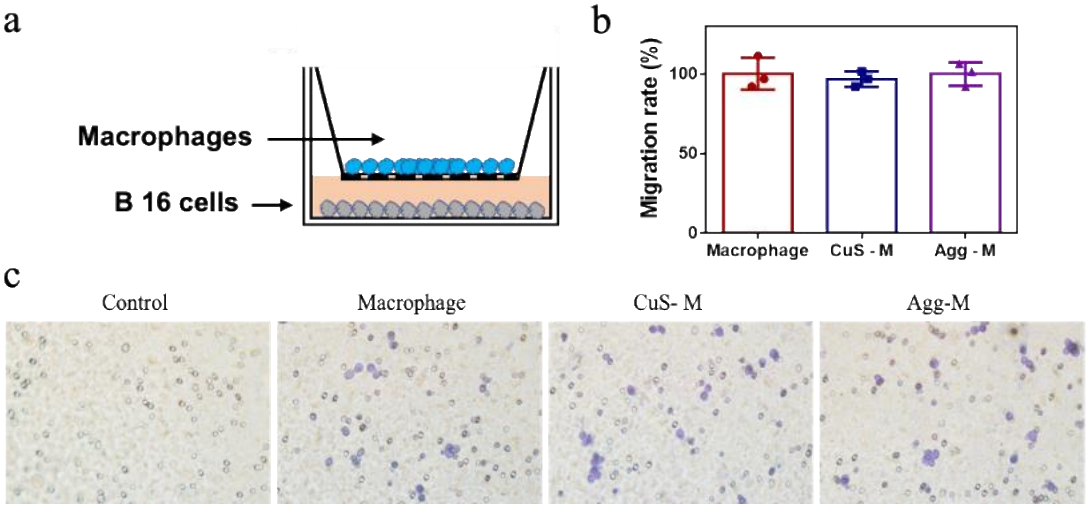


Figure S12. a) Schematic illustration of transwell experiment. b) Quantitative analysis and c) migration ability of macrophage, CuS-M and Agg-M. Blank DMEM served as the negetive control and macrophages served as the positive control.

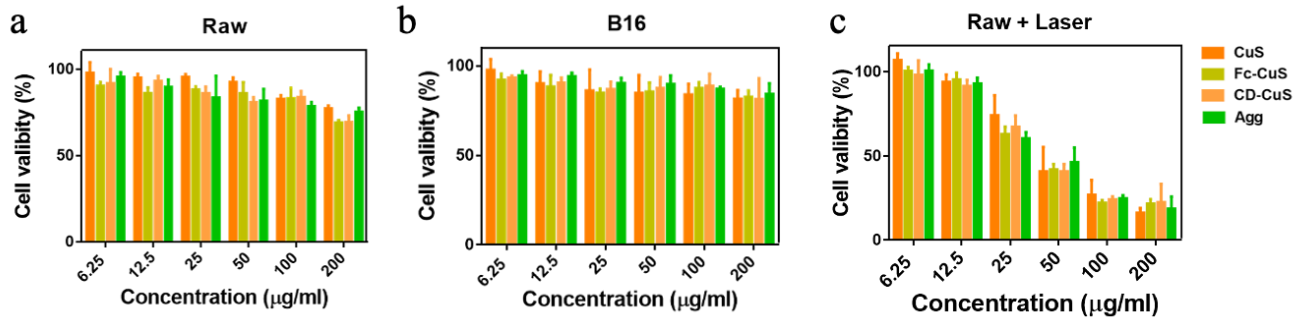


Figure S13. Cell viability of a) macrophages and b) B16 cells incubated with CuS/CD-CuS /Fc-CuS/ Agg at concentrations of 6.25, 12.5, 25, 50, 100 and 200 μg/mL for 24 h. c) Phototoxicity of CuS/CD-CuS /Fc-CuS/ Agg towards macrophages.

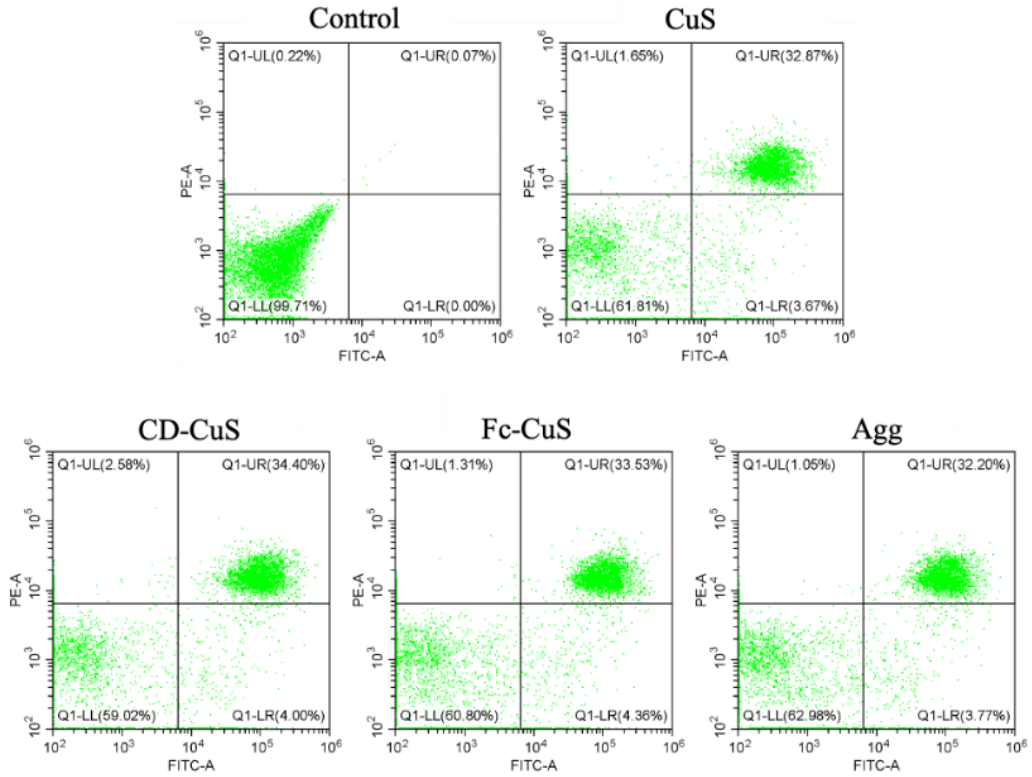


Figure S14. Photothermally induced apoptosis analyzed via flow cytometry after staining with annexin V-FITC/propidium iodide (PI). B 16 cells were treated with CuS/CD-CuS/Fc-CuS/Agg at the concentration of 100 μg/mL, and subjected to 808 laser irradiation for 5 min (1W/cm^2). Blank DMEM served as the control.

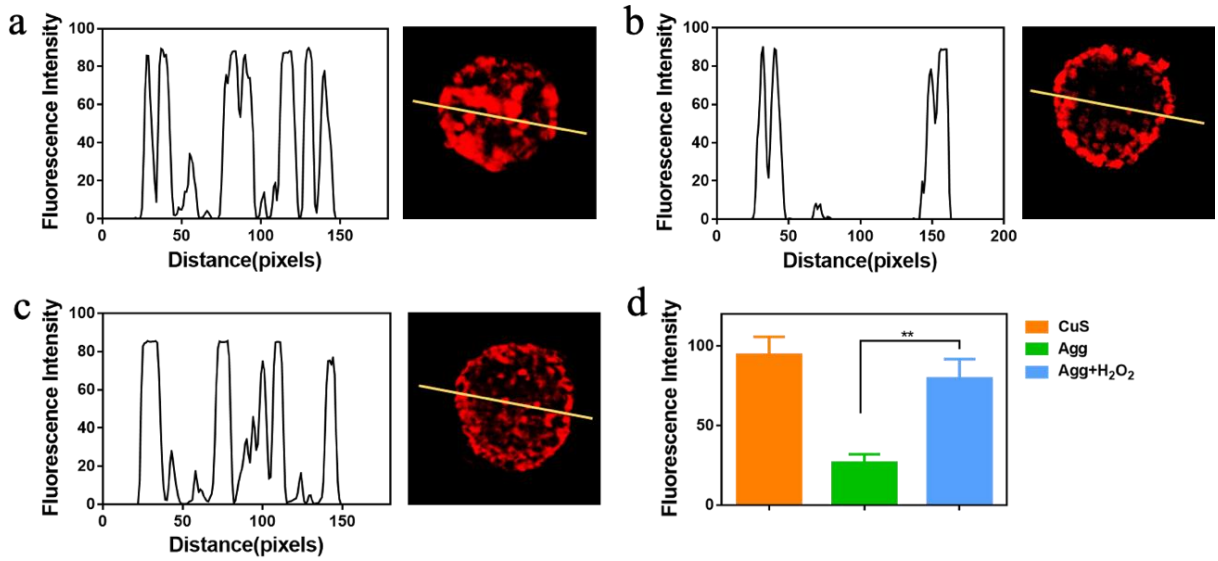


Figure S15. Fluorescence intensity measured from the equatorial plane of tumor spheroid in a) CuS, b) Agg and c) Agg + H_2O_2 group; d) Bar graph quantifying the fluorencene of NPs penetrated into spheroids at the 100 μm depth. All data were presented as mean ± s.d. (n = 3). *P ≤ 0.05, **P ≤ 0.01, and ***P ≤ 0.001.

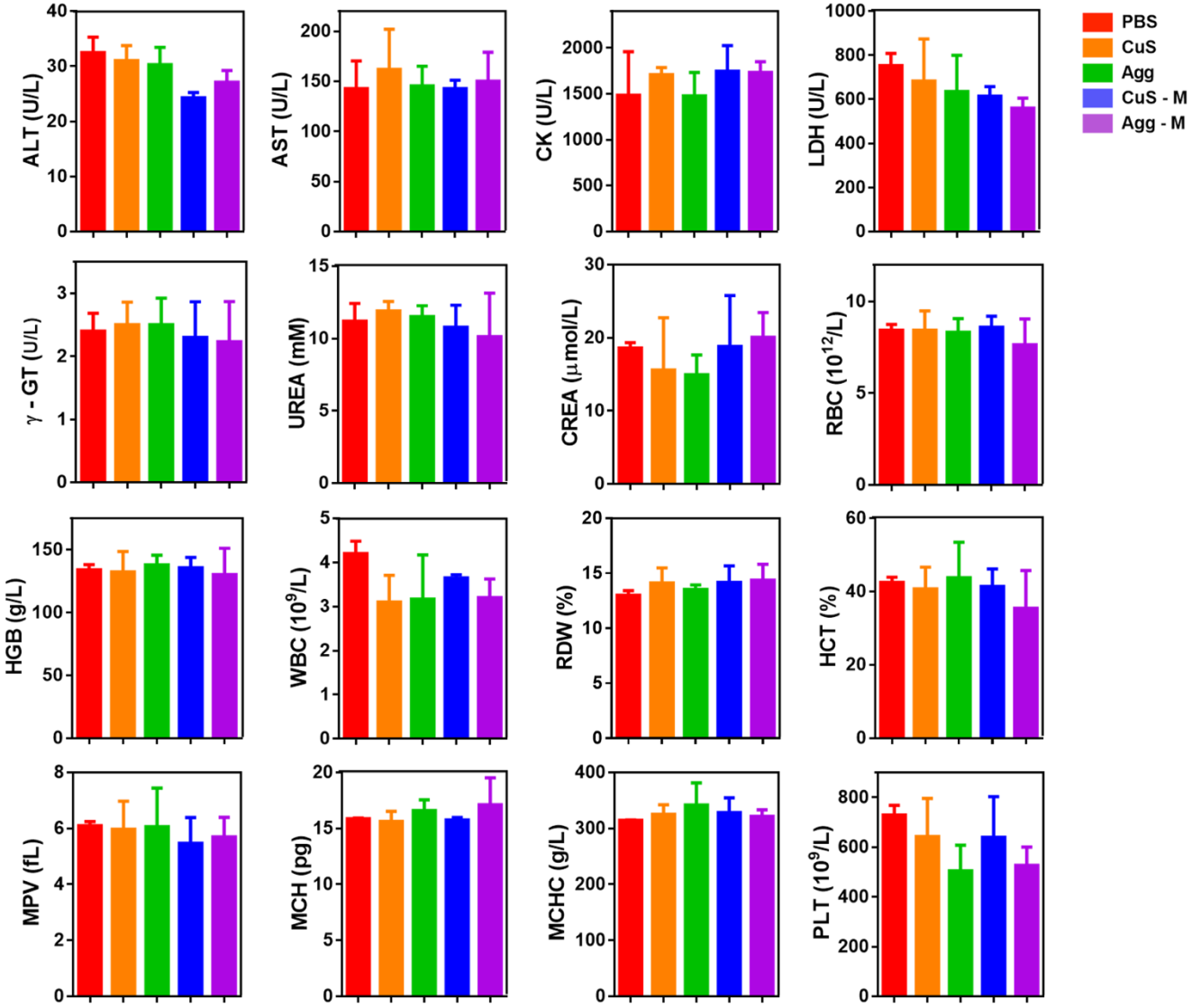


Figure S16. Blood biochemical and hematological parameters of healthy mice intravenously injected with PBS, CuS, Agg, CuS-M and Agg-M 24 h post-injection. ALT: alanine tminotransferase (Normal range: 10.06-96.47 U/L); AST: aspartate aminotransferase(Normal range: 36.31-235.48 U/L); CK: creatine kinase(Normal range: 0-2070.55 U/L); LDH: lactic dehydrogenase(Normal range: 157.41-899.72 /L); γ-GT: gamma-glutamyl transferase(Normal range: 0-7.78 U/L); CREA: creatinine(Normal range: 10.91-85.09 μmol/L); RBC: red blood cells(Normal range: 6.36-9.42 * 10^{12} /L); HGB: hemoglobin(Normal range: 110-143 g /L); WBC: white blood cells(Normal range: 0.8-6.8 * 10^{9} /L); RDW: red cell distribution width(Normal range: 13-17 %); HCT: hematocrit(Normal range: 34.6-44.6 %); MPV: mean platelet volume(Normal range: 3.8-6.0 fL); MCH: mean corpuscular hemoglobin(Normal range: 15.8-19 pg); MCHC: mean corpuscular hemoglobin concentration(Normal range: 320-353 g/L); PLT: platelets(Normal range: 450-1590 * 10^{9} /L).

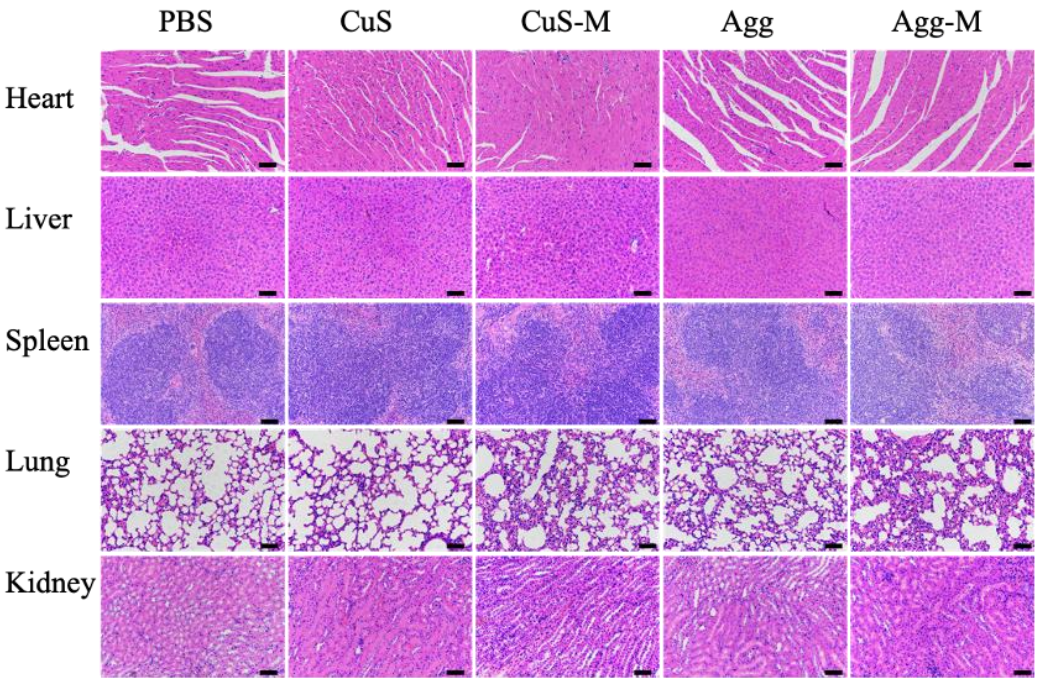


Figure S17. H&E staining of tissue slices from organs collected from healthy mice treated with PBS, CuS, CuS-M, Agg and Agg-M. Scale bar: 10 μm.

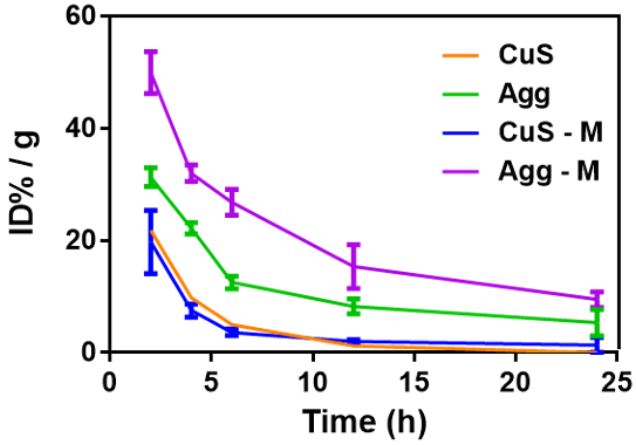


Figure S18. Blood circulation profile of CuS, Agg, CuS-M and Agg-M.

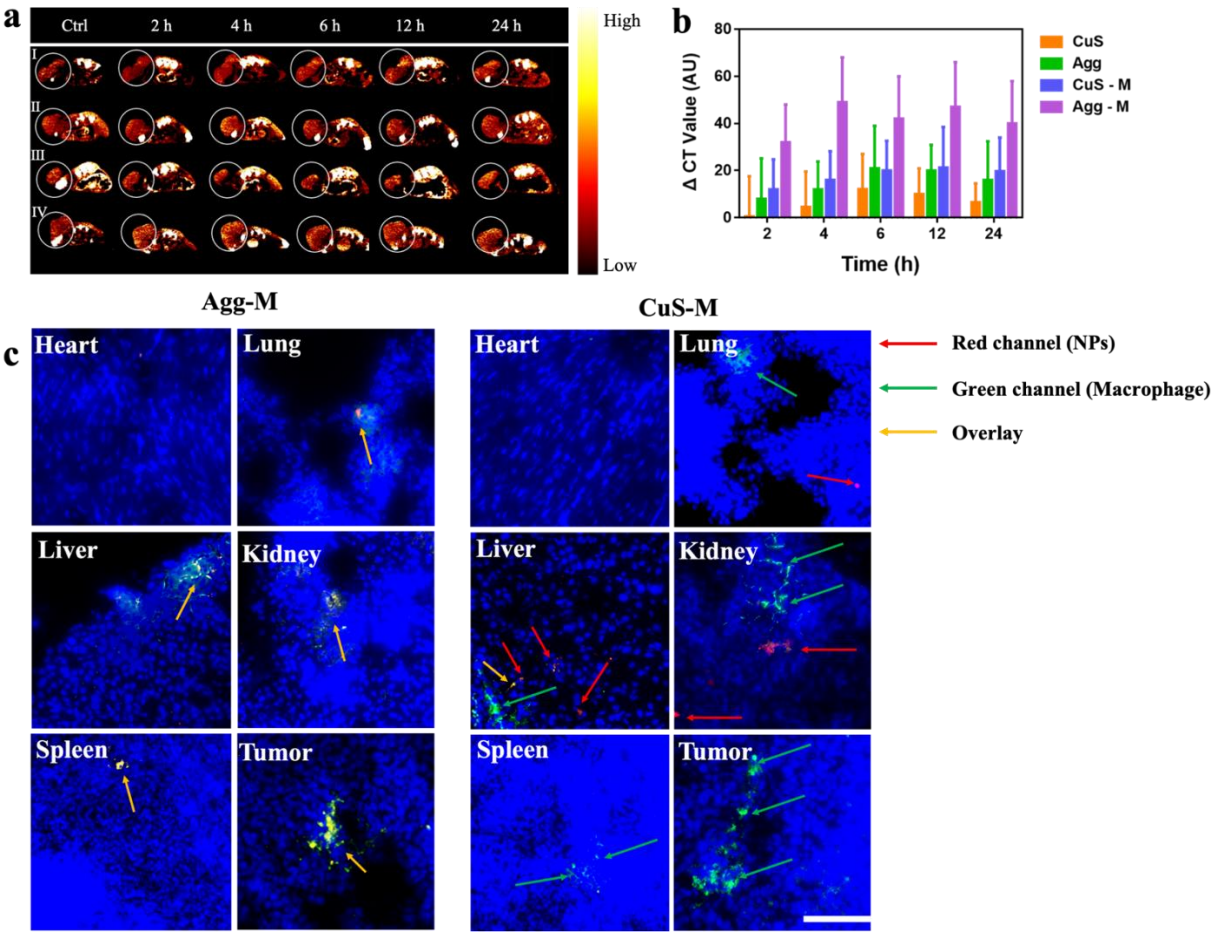


Figure S19. a) Transverse CT images of melanoma-bearing mice injected with I: CuS NPs, II: Agg, III: CuS-M and IV: Agg-M at different time points. Tumor regions were circled with white line. b) Semi-quantitative ROI analysis of the average CT signal in tumors at different time points (n = 3). c) Fluorescent images of tissue sections of the organs and tumors collected from mice treated with CuS-M and Agg-M for 2h. Cy5.5 labelled NPs: red channel; DiI stained macrophage: green channel. Scale bar: 100 μm.

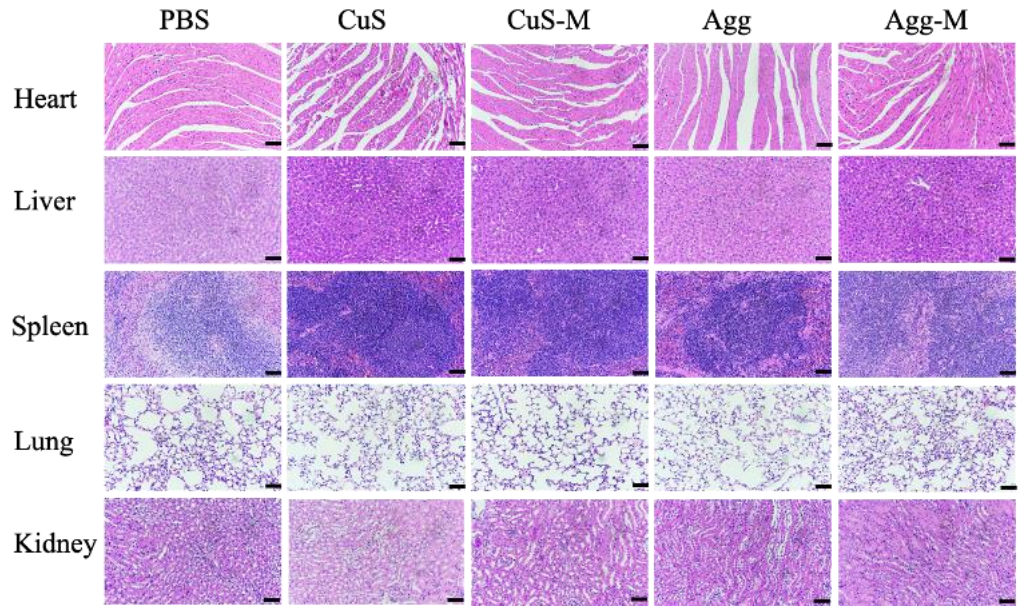


Figure S20. H&E staining of slices from organs collected from melanoma-bearing mice treated with PBS, CuS, CuS-M, Agg and Agg-M. Scale bar: 10 μm.

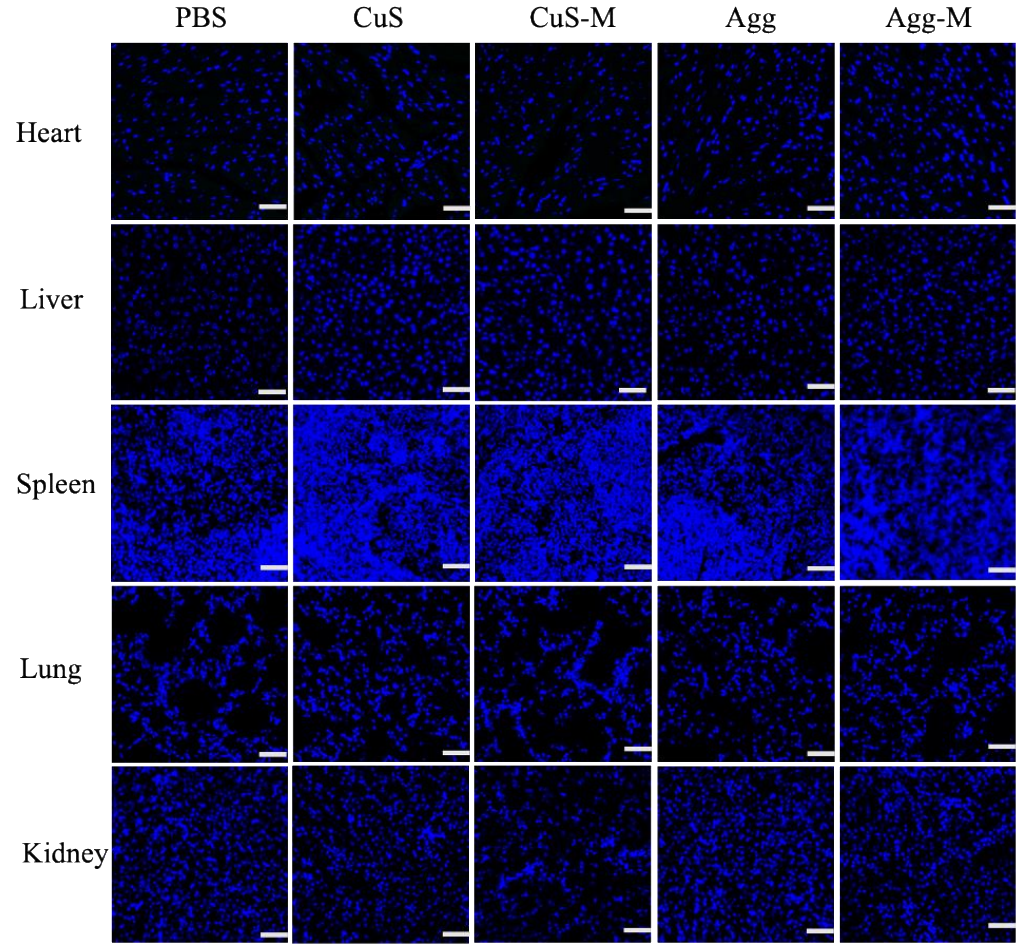


Figure S21. TUNEL staining of slices from organs collected from melanoma-bearing mice treated with PBS, CuS, CuS-M, Agg and Agg-M. The blue and green signals represent DAPI (the nuclei) and apoptotic cells, respectively. Scale bar: 50 μm.

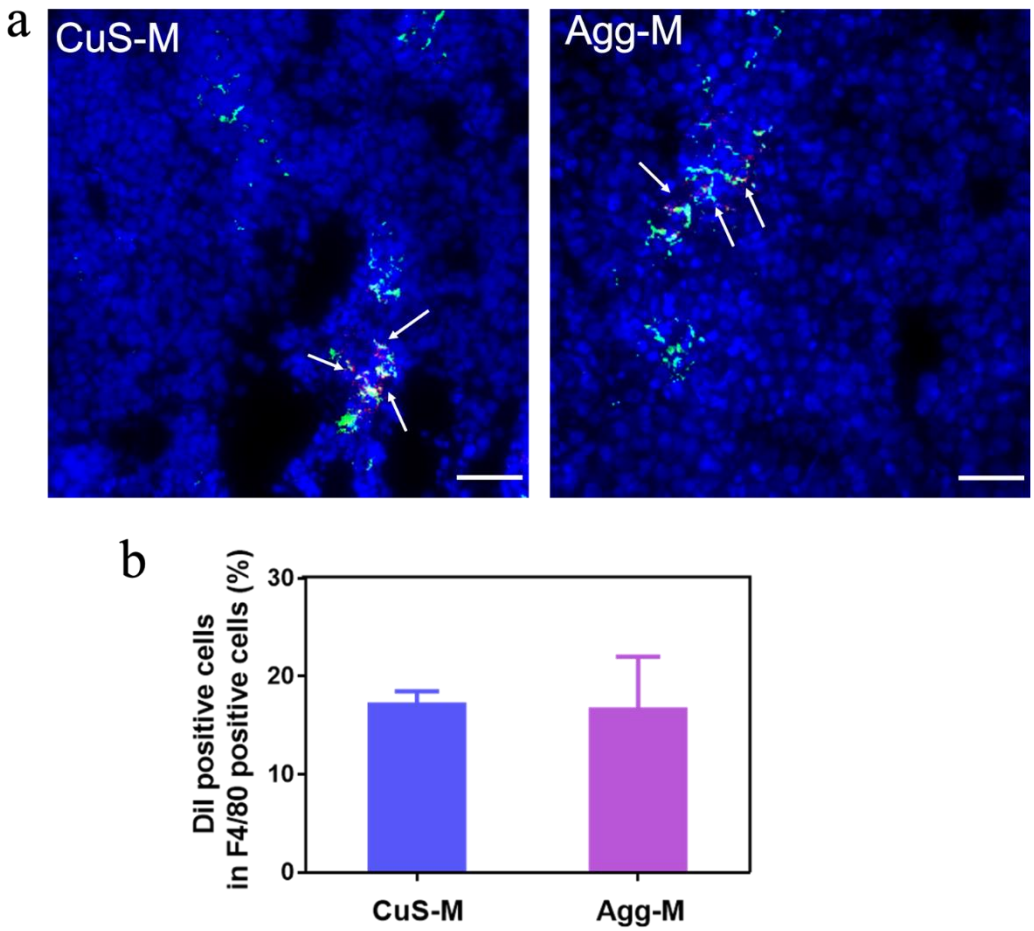


Figure S22. Fluorescent images of the tissue sections of tumors collected from mice treated with CuS-M and Agg-M. Carrier macrophages were stained with DiI (Red channel here) prior to delivery; all macrophages (including major endogenous ones) were stained with FITC labelled F4/80 antibody (Green channel) in the tissue section. Scale bar: 50 μm. b) Quantitative analysis of carrier macrophages (DiI positive cells), relative to all (including endogenous) macrophages (F4/80 positive cells). All data were presented as mean ± s.d. (n = 3).

References

1. M. Zhou, R. Zhang, M. Huang, W. Lu, S. Song, M. P. Melancon, M. Tian, D. Liang and C. Li, *J. Am. Chem. Soc.*, 2010, **132**, 15351-15358.
2. D. Chen, J. Xie, R. Fiskesund, W. Dong, X. Liang, J. Lv, X. Jin, J. Liu, S. Mo, T. Zhang, F. Cheng, Y. Zhou, H. Zhang, K. Tang, J. Ma, Y. Liu and B. Huang, *Nat. Commun.*, 2018, **9**, 873.
3. X. Sun, M. Xu, Q. Cao, P. Huang, X. Zhu and X.-P. Dong, *Sci. Rep.* , 2020, **10**, 1038.
4. D. K. Roper, W. Ahn and M. Hoepfner, *J. Phys. Chem. C*, 2007, **111**, 3636-3641.
5. D. M. Mosser and X. Zhang, *Curr. Protoc. Immunol.*, 2008, **83**, 14.12.11-14.12.18.
6. P. Katila, A. Shrestha, A. Shrestha, R. Shrestha, P.-H. Park and E.-S. Lee, *Bull. Korean Chem. Soc.*, 2018, **39**, 1432-1441.
7. W. Chen, J. Ouyang, H. Liu, M. Chen, K. Zeng, J. Sheng, Z. Liu, Y. Han, L. Wang, J. Li, L. Deng, Y.-N. Liu and S. Guo, *Adv. Mater.*, 2017, **29**, 1603864.