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Rattle-Type Au@Cu_{2-x}S Hollow Mesoporous Nanocrystals with Enhanced Photothermal Efficiency for Intracellular Oncogenic MicroRNA Detection and Chemo-Photothermal Therapy

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Abstract

The coupling of the localized surface plasma resonance (LSPR) between noble metals of Au, Ag and Cu and semiconductors of Cu_{2-x}E ($\text{E} = \text{S}, \text{Se}, \text{Te}$) opens new regime to design photothermal (PT) agents with enhanced PT conversion efficiency. However, it is rarely explored on fabricating of engineered dual plasmonic hybrid nanosystem for combinatory therapeutic-diagnostic applications. Herein, rattle-type $\text{Au}@\text{Cu}_{2-x}\text{S}$ hollow mesoporous nanoparicles with advanced PT conversion efficiency are designed for cellular vehicles and chemo-photothermal synergistic therapy platform. The LSPR coupling between the Au core and Cu_{2-x}S shell are investigated experimentally and theoretically to generate a PT conversion efficiency high to 35.2% and enhanced by 11.3% than that of Cu_{2-x}S . By conjugating microRNA (miRNA) gene probe on the surface, it can realize the intracellular oncogenic miRNA detection. After loading of anticancer drug doxorubicin into the cavity of the $\text{Au}@\text{Cu}_{2-x}\text{S}$, the antitumor therapy efficacy is greatly enhanced in vitro and in vivo due to the NIR photoactivation chemo- and photothermal synergistic therapy. The rattle-type metal-semiconductor hollow mesoporous nanostructure with efficient LSPR coupling and high cargo loading capability will be beneficial to future design of LSPR-based photothermal agents for a broad range of biomedical application.

Keywords

Rattle-type $\text{Au}@\text{Cu}_{2-x}\text{S}$; Localized surface plasma resonance coupling; Chemo-photothermal therapy; MicroRNA detection; Theranostic platform

1. Introduction

Photothermal therapy (PTT) utilizing external light-induced hyperthermia to ablate malignant tissues while avoiding damage to healthy tissue has recently attracted considerable attention for promising clinical applications due to its simplicity, noninvasiveness, safety and remote-controllable properties.[1-5] To achieve effective PTT efficacy, various photothermal (PT) agents such as Au nanostructures, copper sulphide nanoparticles,[6, 7] carbon derivatives, [8-10] transition metal sulfide,[11, 12] black phosphorus,[13, 14] and MXenes [15, 16] are continuously explored. The coupling of the localized surface plasma resonance (LSPR) between noble metal and semiconductor originating from the collective electron and hole oscillations opens new opportunities to optimize PT conversion.[17, 18]

Generally, noble metal such as Au nanostructures support strong LSPR associated with the collective oscillations of the free electrons in the visible spectrum.[13],[19-21] Under resonant excitation, the concentrated free-space optical field within sub-wavelength regions adjacent to Au nanostructures surfaces enable extreme enhancement of electric field, giving rise to the efficient PT conversion with new mechanisms.[22] Cu_{2-x}S NPs is an emerging PT agent sine its near-infrared (NIR) absorption mainly derived from d–d energy band transition of Cu^{2+} ions and not affected by its morphology and environments.[23, 24] Importantly, Cu_{2-x}S NPs with numerous copper-deficiency possess large amount of p-type hole carriers with high mobility and support LSPR in the near-infrared (NIR).[25, 26] Therefore, integration of the two kinds of plasmonic nanostructure in a single unit can produce fascinating properties due to the synergistic effect. Jiang et al constructed a dual plasmonic hybrid nanosystem Au- Cu_9S_5 nanosystem with enhanced light adsorption for robust PTT in the second NIR window.[27] Zhang et al fabricated a monodisperse structurally

well-defined $\text{Au}@\text{Cu}_{2-x}\text{S}$ with well LSPR coupling of Au rod and Cu_{2-x}S for enhanced the PT conversion efficiency and heightened the PTT effect. [28] Among a diverse set of Cu_{2-x}S nanostructures, hollow Cu_{2-x}S nanospheres have particular interests for chemo-photothermal therapy due to the high drug loading capacities, which have never been explored for the $\text{Au}@\text{Cu}_{2-x}\text{S}$ plasmonic hybrid nanosystem.

Moreover, integrating diagnostic and therapeutic functions in one single nanostructure to develop theranostic platform has attracted intense attention in the personalized nanomedicine and clinical application. MicroRNA (miRNAs) are single-stranded, short (approximately 19–23 nucleotides), endogenous, noncoding RNAs that play a key role in various biological and pathological processes.[29, 30] The dysregulated expression of miRNAs is associated with various tumors, and miRNAs are emerging as significant diagnostic markers and therapeutic targets for tumor cells.[31-33] Thus the theranostic nanoplatform with the ability for intracellular oncogenic miRNA detection provided great promise for clinical diagnosis and therapeutic.

Herein, a novel rattle-type $\text{Au}@\text{Cu}_{2-x}\text{S}$ hollow dual plasmonic nanosystem with an independent Cu_{2-x}S shell and optimized size of Au cores are designed as multifunctional theranostic platform. Optimized LSPR coupling between the Au core and Cu_{2-x}S shell are realized by experimental investigation and theoretical calculation. The generated engineered $\text{Au}@\text{Cu}_{2-x}\text{S}$ displayed much higher PT conversion efficiency than the pure Cu_{2-x}S agent due to the LSPR coupling. The unique rattle-type hollow mesoporous structures allow versatile surface bioconjugation and drug loading for delivering gene probe for intracellular oncogenic miRNA detection, which have never been explored for $\text{Au}@\text{Cu}_{2-x}\text{S}$ structure. It realizes greatly enhanced antitumor efficacy by combing chemo- and photothermal therapy.

2. Materials and methods

2.1 *Materials and reagents*

Terminal sulfydryl polythylene glycol (PEG-SH) was synthesized by Sinopeg Biotech Co., Ltd (Xiamen, China). Poly(vinylpyrrolidone) (PVP-K30), cupric chloride (CuCl_2), sodium sulfide (Na_2S) and hydrazine anhydrous were obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). MTT and HAuCl_4 were purchased from Sigma-Aldrich (China). All other reagents were of analytical grade. Hoechst 33342, calcein-AM and propidium iodide (PI) were obtained from Yeasen Biotech. Co., Ltd. (Shanghai, China). The oligonucleotides were purchased from Sangon Biological Engineering Technology & Co., Ltd (Shanghai, China) and purified using high-performance liquid chromatography.

2.2 *Characterization Instrument*

The morphologies of $\text{Au}@\text{Cu}_{2-x}\text{S}$ were examined with a FEI20 transmission electron microscope (TEM) (FEI, USA) and a JSM-6700F scanning electron microscope (SEM) (JEOL, Japan). The UV–visible (UV–vis) absorption analysis was recorded with an UV-1800 spectrophotometer (Shimadzu, Japan). Zeta potential analysis was performed on Nano ZS (Malvern, UK). X-ray diffraction (XRD) pattern of the as-prepared materials was recorded by a Bruker-AXS X-ray diffractometer with $\text{Cu K}\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$). All fluorescence measurements were performed on a confocal laser scanning fluorescence microscope (CLS, FV1200, Olympus, Japan).

2.3 *Synthesis of Gold Nanoparticles*

The AuNPs of different sizes were synthesized according to the previous report

with some modification.[34] A solution of 2.2 mM sodium citrate was boiled in a 250 mL three-necked round-bottomed flask for 15 min under vigorous stirring with a condenser to prevent the evaporation of the solvent. 1 mL of HAuCl₄ (25 mM) was injected and kept the reaction for 10 min until the color of the solution changed to soft pink to obtain the Au seeds. The solution was cooled to 90 °C, then 1 mL of sodium citrate (60 mM) and 1 mL of HAuCl₄ solution (25 mM) were sequentially injected. 10 mL of solution were extracted in 30 min, 1 mL of HAuCl₄ solution (25 mM) was injected and repeat the operation in the next 30 min to generate AuNPs with the size of 20 nm and 30 nm, respectively. The 55 mL of final sample was diluted by 53 mL of pure water and 2 mL of sodium citrate (60 mM) to obtain the seed solution of AuNPs. The seed solution was reacted at 90 °C, then 1 mL of HAuCl₄ solution (25 mM) was added. 10 mL of solution (40 nm) were extracted in 30 min and another 1 mL of HAuCl₄ solution (25 mM) was injected, the solution was reacted for another 30 min to generate AuNPs with size of 50 nm, respectively.

2.4 Fabrication of Au@Cu_{2-x}S Hollow Spheres

Au@Cu_{2-x}S hollow spheres were synthesized according to previously reported method with some modification.[35-37] Briefly, CuCl₂ solution (100 μL, 0.5 mol/L) was added to deionized water (25 mL) containing poly(vinylpyrrolidone) (PVP-K30, 0.24 g) in a round flask under stirring at room temperature, followed by adding 1mL AuNPs (0 mM) with different sizes and 25 mL NaOH solution (pH 9.0) was added and reacted for 2 min. 6.4 μL hydrazine anhydrous solution was added into the solution to form Cu₂O spheres, and the color of the solution changed to yellow green. After 5 min, Na₂S aqueous solution (200 μL, 320 mg/mL) was added to the round flask and reacted at 75°C for 2 h, the color of the solution changed to dark brown. The

as prepared Au@Cu_{2-x}S hollow spheres were centrifuged at 11000 rpm for 15 min and washed twice with distilled water.

2.5 Functionalization, Gene and Drug Loading of Au@Cu_{2-x}S

1.5 mg SH-PEG (M_w = 2 k Da) was added into 1 mL the as-prepared Au@Cu_{2-x}S hollow spheres and stirred for 12 h at room temperature. The product was centrifuged and washed three times to remove the unbounded SH-PEG for obtaining Au@Cu_{2-x}S-PEG.

For miRNA gene probe loading, S1 chain and S2 chain were first hybridized at 37 °C for 1 h to gain S1/S2 duplex structure. Then the detection duplex probe was added to Au@Cu_{2-x}S-PEG solution and stirred for 12 h at room temperature. The product was centrifuged and washed three times to remove the unbounded detection duplex probe to obtain DP-Au@Cu_{2-x}S-PEG.

1 mg Au@Cu_{2-x}S-PEG was added to 500 µL DOX solution at different concentration (100, 200, 400, 600, 1000 µg/mL), and the mixture was shaken for 24 h in the dark at room temperature. Then, the as-prepared Au@Cu_{2-x}S/DOX-PEG were separated from free DOX solution by centrifugation at 10000 rpm for 10 min and washed with PBS (pH 7.4, 10 mM). The amount of the unloaded DOX remaining in the supernatant was quantified by the UV-vis adsorption at 480 nm with UV-vis spectrophotometer (Shimadzu, Japan), based on which the drug loading capacity was calculated.

To investigate the photoresponsible drug release efficiency of the nanocarrier, Au@Cu_{2-x}S/DOX-PEG (1 mg) were dispersed in 1 mL PBS (10 mM) at pH of 7.4 and 5.0, respectively. After irradiating with 980 nm NIR laser (0.72 W cm⁻²) for different times, the supernatant was taken out by centrifugation at 11000 rpm. DOX

concentration in the withdraw solution was quantified by the UV-vis adsorption at 480 nm with UV-vis spectrophotometer.

2.6 *PT Properties Characterization of Au@Cu_{2-x}S*

The PT performance of Au@Cu_{2-x}S hollow spheres were analyzed by irradiating a quartz cuvette containing aqueous dispersion of Au@Cu_{2-x}S hollow spheres at different concentrations (10, 20, 50, 100, 200 µg/mL) with a 980 nm multimode pump laser (Hi-Tech Optoelectronics Company) at a power of 0.72 W/cm². The temperature of the aqueous dispersion was measured and recorded by an OMEGA 4-channel datalogger thermometer. To investigate the photothermal stability of Au@Cu_{2-x}S, Au@Cu_{2-x}S (100 µg/mL) were irradiated at 980 nm (0.72 W cm⁻², 10 min; laser on), followed by cooling to room temperature without irradiation (laser off). The laser on/off cycles were repeated four times for temperature monitoring.

2.7 *Cytotoxicity Experiments*

MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 1% penicillin and streptomycin at 37 °C in a humidified atmosphere containing 5% CO₂. MCF-7 cells (1.0×10^5 cells per well) were first cultured for 24 h in a 96-well plate containing DMEM with 10% FBS (100 µL) in each well, and then the medium was replaced with fresh Opti-MEM alone or medium containing Au@Cu_{2-x}S-PEG at different concentrations (10, 25, 50, 100 and 200 µg/mL) and incubated for another 4 h. Afterward, the cells were cultured for another 12 h. Then, fresh DMEM (100 µL) containing 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) (10 µL, 5 mg/mL) were then added to each well. The media were removed 4 h later,

and dimethylsulfoxide (DMSO) (100 μ L) was added to solubilize the crystal violet. After shocking for 15 min, the absorbance of each well was measured using an Anthos 2010 microplate reader (biochrom, USA) at 492 nm. The cytotoxicity of Au@Cu_{2-x}S and Au@Cu_{2-x}S/DOX-PEG was investigated in the same procedure.

2.8 Intracellular miRNA Detection

Detection probe for miRNAs-155:

S1: FAM-ACC CCT ATC ACG ATT AGC ATT AA

S2: SH-AAAAAAA AAA A-(BHQ)-TG GGG ATA GTG CTA A

MCF-7 cells (1.0×10^4) were cultivated in confocal dish containing DMEM (1 mL) for 12 h. The medium was then replaced with fresh Opti-MEM (1 mL) containing Au@Cu_{2-x}S-PEG (100 μ g/mL, 50 nM loaded miRNA detection probe) and cultivated for 4 h. After washing each dish twice by PBS (pH 7.4, 10 mM), the fresh DMEM medium (1 mL) was added and cultured for another 12 h. After dyeing the cell nucleus with Hoechst 33342, and the cells were imaged using a CLSM.

2.9 In Vitro Chemo-Photothermal Dual-Modal Therapy

MCF-7 cells (5.0×10^5) in 1 mL DMEM were incubated with Au@Cu_{2-x}S-PEG or Au@Cu_{2-x}S/DOX-PEG (100 μ g/mL with loading 20 μ g/mL DOX) or PBS (pH 7.4, 10 mM) at 4 °C for 1 h, and then irradiated for 10 min with a 980 nm laser at a power of 0.72 W/cm². The cells were then immediately plated in a 96-well plate containing 100 μ L of DMEM media. After incubated for 24 h, the cell viability was determined by MTT assay. After dyeing the cell with calcein-AM (live: green) and propidium iodide (PI, dead: red), the cells were imaged using a CLSM.

2.10 *In Vivo Combined Chemo and Photothermal Therapy*

Female BALB/c nude mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The Institutional Animal Care and Use Committee of the Beijing institute of Basic Medical Science (Beijing, China) approved all the animal experiments. To obtain tumor-bearing animals, 5×10^6 A549 cells were suspended in 100 μ L PBS and subcutaneously injected into right back flank of nude mice. Animals were maintained at a temperature of $24 \pm 2^\circ\text{C}$ with a normal day-night cycle and free access to water and food. When the tumor volume reached about 100 mm^3 , BALB/c tumor-bearing nude mice were randomly divided into 3 groups ($n=3$, in each group), which were injected with PBS (pH 7.4, 10 mM), Au@Cu_{2-x}S-PEG, Au@Cu_{2-x}S/DOX-PEG (100 $\mu\text{g/mL}$) at the tumor sites, respectively. Then, they were irradiated with 980 nm laser for 5 min. During the irradiation, an infrared thermal camera (Fluke, USA) was used to monitor the temperature changes of the tumor sites. Tumor size and body weight of the mice were measured using a caliper and an electronic balance, respectively. Additionally, H&E staining was performed to observe the tissue destruction after therapy. Cell proliferation within tumors was evaluated by immunostaining against ki-67.

3. Results and Discussion

3.1 Preparation and Characterization of Au@Cu_{2-x}S

The core-shell Au@Cu_{2-x}S were synthesized through a modified Kirkendall process by using Au@Cu₂O as sacrificial template to replace oxygen ions with sulfur ions[38] (**Figure 1a**). Transmission electron microscopy (TEM) measurements were used to analyze the nanoparticles (NPs) generated during the Au@Cu_{2-x}S fabrication

process. The wine red Au NPs with desired size were first synthesized (**Figure 1b**), then a thin layer of Cu₂O was controllably grew on the surface of Au NPs to form uniform Au@Cu₂O core-shell NPs with an average diameter of about 106 nm (**Figure 1c**). The high-resolution TEM (HRTEM) of Au@Cu₂O (**Figure 1e**) revealed the lattice constant of 0.296 nm and 0.246 nm, corresponding to the (110) and (111) planes of Cu₂O shell.[39, 40] The addition of S²⁻ enabled the transformation of light yellow Au@Cu₂O into larger size and dark green Au@Cu_{2-x}S hollow mesoporous spheres with mean diameter of about 150 nm through Kirkendall effect (**Figure 1d**). Herein, the Cu²⁺ to S²⁻ ratio is close to Cu_{1.81}S, as confirmed by energy dispersive spectroscopy (EDS) in **Figure S1a**, which accorded with X-ray diffraction (XRD) pattern data and illustrated the non-stoichiometry of Cu_{2-x}S shell. The lattice constant of 0.276 nm and 0.302 nm corresponding to the (124) and (115) planes of Cu_{2-x}S were clearly observed on the ligaments in the HRTEM of Au@Cu_{2-x}S (**Figure 1f**), which was verified by the XRD pattern analysis (**Figure S1b**). The UV-spectra (**Figure S1c**) and dynamic light scattering (DLS) (**Figure S1d**) characterizations further confirmed the successful synthesis of Au@Cu_{2-x}S hollow mesoporous spheres from Au NPs. **Figure 1g** illustrated the scanning electron microscope (SEM) images of the Au@Cu_{2-x}S hollow spheres, revealing the surface of the hollow sphere was irregular and interspersed with numerous mesoporous. The average pore diameter was determined to be 3.244 nm and the Brunauer–Emmett–Teller (BET) surface area was calculated as 21.11 m²/g by N₂ adsorption-desorption measurement (**Figure S2**).

Figure 1. a) Scheme illustration of the synthesis process of the Au@Cu_{2-x}S hollow mesoporous spheres; TEM images of b) Au NPs, c) Au@Cu₂O core-shell NPs and d) Au@Cu_{2-x}S hollow mesoporous spheres; Insert (b-d) were photographs of HRTEM images of Au NPs, Au@Cu₂O and Au@Cu_{2-x}S solutions; e) Au@Cu₂O core-shell NPs and f) Au@Cu_{2-x}S hollow mesoporous spheres; g) SEM image of Au@Cu_{2-x}S hollow mesoporous spheres.

3.2 Enhanced Photothermal Property of Au@Cu_{2-x}S

The Au@Cu_{2-x}S was designed to study surface-enhanced near-field of Au NPs on the collective hole oscillation of neighboring Cu_{2-x}S NPs for construct photoabsorbers with high PT conversion efficiency due to the LSPR coupling (**Figure 2a**). In the proposed rattle-type Au@Cu_{2-x}S nanostructure, the size and Cu_{2-x}S shell thickness was constant, therefore, the size of Au NPs core was optimized to maximize the PT conversion efficiency. The Au NPs with different sizes (20, 30, 40, 50 nm) were firstly synthesized[34] and characterized by the TEM and UV-vis analyses (**Figure S3**), and the corresponding Au@Cu_{2-x}S were denominated Au@Cu_{2-x}S-20, Au@Cu_{2-x}S-30,

Au@Cu_{2-x}S-40, and Au@Cu_{2-x}S-50, respectively (**Figure 2b**).

As shown in **Figure 2c-g**, the Cu_{2-x}S presented lower temperature increase than these Au@Cu_{2-x}S NPs at the same condition, and Au@Cu_{2-x}S NPs displayed a core size-dependent temperature increase pattern. The Au@Cu_{2-x}S with larger Au core size displayed a quicker and larger temperature increase compared to these with smaller Au core size, and the Cu_{2-x}S hollow spheres presented an inefficient temperature increase compared to these Au@Cu_{2-x}S NPs counterparts (**Figure S4**). It indicated that the coupling between collective electron and hole oscillations increased along with the increase of the core size, leading to enhancement of Cu_{2-x}S shell plasmonic absorption and high PT conversation efficiency. The PT conversion efficiency of the optimized Au@Cu_{2-x}S-50 structure was calculated to 35.2%^[19] (**Figure S5**), which was higher than that of pure Cu_{2-x}S and other three Au@Cu_{2-x}S NPs (**Figure S6**) and superior to that of pure copper sulfide nanoparticles like Cu_{2-x}S nanocrystals (16.3%), Cu_{2-x}Se nanocrystals 22% and Cu₉S₅ nanocrystals 25.7% and pure gold materials like Au nanocage (27.9%) and Au nanorods (21%) in the previous reports.^[41-44] Therefore, the Au@Cu_{2-x}S-50 was selected in the following experiments. To explore the PT stability, the Au@Cu_{2-x}S solution was irradiated with NIR laser for 10 min (laser on), followed by naturally cooling to room temperature upon the switch off the NIR laser (laser off) and then repeated the cycle of laser on/off. It illustrated good stability and excellent reproducibility of Au@Cu_{2-x}S in these cycles (**Figure 2h**, **Figure S7**).

To explore the possible contribution of local field enhancement to the photothermal properties of Au@Cu_{2-x}S hollow spheres, theoretical simulation using MIT Electromagnetic Equation Propagation (Meep) was performed.^[45-47] Meep is an open-sourced simulation package that adopt finite difference time domain (FDTD)

to solve Maxwell equations. The simulation region is a cuboid space, the edge-length of this region is 220 nm, and the optical media inside is water, with its permittivity equals to 1.7. A plane wave source whose wavelength equals to 980 ± 0.2 nm has been placed inside the simulation region, and the size of the light source is $220 \text{ nm} \times 220 \text{ nm}$. The mesh of our simulation region was set to be 1.0 nm in each direction, and the Courant factor was 0.5. The permittivity of both Cu_{2-x}S and Au are expressed by Lorentz model, as is shown below,

$$\varepsilon(\omega) = 1 + \sum_{i=1}^N \frac{A_i \omega_i^2}{\omega_i^2 - \omega^2 - i\gamma_i \omega}$$

For Cu_{2-x}S , two Lorentzian term are needed, thus $N=2$, with parameters achieved by fitting the extinction spectrum of pure Cu_{2-x}S hollow structure. For Au, six Lorentzian terms were used to fit the published data in 1972 from Johnson and Christy.[48] As shown in **Figure 2i**, the electric field enhancement of Cu_{2-x}S , $\text{Au@Cu}_{2-x}\text{S-20}$, $\text{Au@Cu}_{2-x}\text{S-30}$, $\text{Au@Cu}_{2-x}\text{S-40}$, and $\text{Au@Cu}_{2-x}\text{S-50}$ were calculated with the electric field distribution at 980 nm. A strong electric field enhancement was observed in the junction of Au core and Cu_{2-x}S shell due to the LSPR coupling compared to single Cu_{2-x}S , and the increase of LSPR coupling along with the increase of the Au core, which was in agreement with the experimental results.

Figure 2. a) Scheme diagram of the coupling effect of LSPR; b) Schematic illustration and TEM images of Au@Cu_{2-x}S hollow mesoporous spheres with different Au core sizes (0, 20, 30, 40, 50 nm), scale bar: 50 µm; c-g) PT heating curves of Cu_{2-x}S hollow mesoporous spheres and different Au@Cu_{2-x}S NPs at different concentration (0, 10, 25, 50, 100, and 200 µg/mL); h) The PT conversion cycling test of Au@Cu_{2-x}S hollow mesoporous spheres solution (100 µg/mL) under 0.72 W cm⁻², 980 nm laser irradiation; i) Calculated electric field distribution of Cu_{2-x}S, Au@Cu_{2-x}S-20, Au@Cu_{2-x}S-30, Au@Cu_{2-x}S-40, and Au@Cu_{2-x}S-50 at 980 nm.

3.3 Intracellular miRNA Detection of Au@Cu_{2-x}S

The unique hollow mesoporous spheres structure of Au@Cu_{2-x}S allowed efficient gene probe and drug loading for diagnosis and synergetic therapy, which were illustrated in **Figure S8**. Using oncogenic miRNA-155 as model, the detection

duplex probe was conjugated to Au@Cu_{2-x}S functionalized with polyethylene glycol (PEG) (DP-Au@Cu_{2-x}S-PEG) through Cu-S bonds for intracellular miRNA detection (**Figure S9a**). The quencher in the S2 chain extinguished the fluorescence of S1 in absence of target (**Figure 3a**). After the DP-Au@Cu_{2-x}S-PEG were transfected into MCF-7 cells, the S1 chain hybridized with intracellular miRNA-155 and dissociated from the S2 chain to produce green fluorescence signal for detection. As shown in **Figure 3b**, the Hoechst 33342 dyed the cell nucleus to blue color, in comparison with weak green fluorescence in normal human dermal fibroblasts (nHDF) cells with low expression of miRNA-155, stronger green fluorescence signal was observed in MCF-7 cancer cells with high expression of miRNA-155. The fluorescence intensity analysis of MCF-7 and nHDF cells further clearly revealed the higher expression level of cancer-related miRNA-155 in the cytoplasm of MCF-7 cancer cells than that of the nHDF cells (**Figure 3c,d**), providing an efficient cancer diagnostic tool.

Figure 3. a) Scheme diagram of the process for intracellular miRNA detection; b) Confocal laser scanning microscope (CLSM) images of MCF-7 cells and nHDF cells transfected by DPAAu@Cu_{2-x}S-PEG-P (100 μg/mL, 50 nM linked detection probes). Scale bars: 40 μm. c, d) Fluorescence intensity of MCF-7 and nHDF cells. Blue: cell nucleus, green: FAM.

3.4 Drug Loading and Photoresponsible Release of Au@Cu_{2-x}S

Doxorubicin (DOX) was loaded onto Au@Cu_{2-x}S-PEG to fabricate a chemo-photothermal dual-modal therapeutic platform (Figure S9). The loading capacity was quantified to be as high as 908 μg DOX per mg of Au@Cu_{2-x}S-PEG (Figure 4a), which is higher than previous reports [49]. As shown in Figure 4b, the release of DOX from Au@Cu_{2-x}S/DOX-PEG presented a pH-related and photo-responsible drug release pattern. The drug release was mainly

photo-responsible and pH also contributed to the drug release. In this system, most of DOX was loaded into cave and pore of the hollow mesoporous nanospheres, the local hyperthermia accelerate diffusion rate of the DOX from the Au@Cu_{2-x}S. The weak acid environment induced increased solubility and also increased diffusion rate. The release rate at pH 5.0 was almost 2-folds higher than that at pH 7.4 in 20 min without NIR laser irradiation, which is beneficial to intratumoral drug delivery. Under irradiated by NIR laser, a dramatic release were observed for both pH condition, and over 70% DOX was released in 20 min, indicating a photoresponsible release behavior.

As shown in **Figure 4c**, the Au@Cu_{2-x}S/DOX-PEG incubated MCF-7 cells without NIR laser irradiation presented weak red fluorescent signal of DOX, suggesting slight release of DOX. On the contrary, strong red fluorescence was observed when the Au@Cu_{2-x}S/DOX-PEG-treated MCF-7 cells exposed to NIR laser irradiation for 5 min, and much stronger fluorescence was presented in the Au@Cu_{2-x}S/DOX-PEG-treated MCF-7 cells irradiated for 10 min. As control, weak red fluorescence was observed in the cells incubated for 5 min or 10 min without irradiation (**Figure S10**). The intracellular fluorescence intensity analysis further clearly demonstrated the photoresponsible intracellular DOX release behavior (**Figure S11**). The MCF-7 cancer cells derived multicellular tumor spheroids (MCTSs) were employed as a mimicking *in vivo* model to assess the photoresponsible release capacity of Au@Cu_{2-x}S/DOX-PEG (**Figure 4d**). The red fluorescence of DOX was clearly observed even at the scanning depth of 105 μm , demonstrating remarkable drug delivery and photoresponsible release ability. The photoresponsible release ability in deep tissue is vital for improved therapy efficiency.

Figure 4. a) DOX loading capacity of Au@Cu_{2-x}S-PEG at various DOX concentrations. b) DOX releasing from Au@Cu_{2-x}S/DOX-PEG with or without 980 nm laser irradiation. c) CLSM images of Au@Cu_{2-x}S/DOX-PEG-treated MCF-7 cells without and with 980 nm laser irradiation. Scale bar: 40 μm. d) CLSM images of Au@Cu_{2-x}S/DOX-PEG-treated MCF-7 MCTSs with 980 nm laser irradiation for 10 min. Scale bar: 200 μm.

3.5 *In vitro* Chemo-Photothermal Dual-Modal Therapy

The superb PT conversion efficiency and conspicuous capability for drug loading and photoresponsible release make the Au@Cu_{2-x}S-PEG a promising agent for chemo-photothermal dual-modal therapy. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay and calcein-AM/propidium iodide (PI) staining were used to investigate the combined therapeutic efficiency. The Au@Cu_{2-x}S-PEG NPs displayed slight cytotoxicity in absence of NIR-laser irradiation (**Figure**

5a). As shown in **Figure 5b**, neither Au@Cu_{2-x}S-PEG (Au@Cu_{2-x}S 100 µg/mL) nor Au@Cu_{2-x}S/DOX-PEG (Au@Cu_{2-x}S 100 µg/mL loaded with 20 µg/mL DOX) without NIR laser irradiation induced significant cytotoxicity, indicating good biocompatibility of Au@Cu_{2-x}S-PEG and Au@Cu_{2-x}S/DOX-PEG. Under NIR-laser irradiation, the Au@Cu_{2-x}S-PEG treated MCF-7 cells exhibited a cell death ratio of 74.78%, significantly higher than 7.50% of the cells transfected with PBS (10 mM, pH 7.4) and 30.20% of DOX (20 µg/mL DOX)-treated cells. Notably, the Au@Cu_{2-x}S/DOX-PEG incubated MCF-7 cells irradiated with NIR-laser presented a cell viability ratio down to 9.75%, which was much lower than the 25.22% of Au@Cu_{2-x}S PTT and 69.80% of DOX chemotherapy, indicating the excellent tumor inhibitory rate of the combined chemo-/phototherapy treatment. Meanwhile, the loaded of DOX into Au@Cu_{2-x}S effectively reduced the side effect of DOX due to the photoresponsible release behavior.

The Calcein-AM/ PI staining analysis further confirmed the remarkable chemo-photothermal therapy efficacy (**Figure 5c**). Under NIR irradiation, the Au@Cu_{2-x}S/DOX-PEG-treated MCF-7 cells showed much higher cell death ratio than Au@Cu_{2-x}S-PEG treated MCF-7 cells and DOX-treated cells with or without NIR irradiation, indicating the distinctive synergetic therapy efficiency of chemo-photothermal dual-modal therapy. The feasibility of the in vivo chemo-photothermal dual-modal therapy capability of Au@Cu_{2-x}S/DOX-PE was first evaluated using MCTSs. The DOX-treated MCTSs and Au@Cu_{2-x}S/-PEG-treated MCTSs irradiated with NIR laser presented higher death ratio than control MCTSs, while the Au@Cu_{2-x}S/DOX-PEG-treated MCTSs were almost completely apoptotic and necrosis under irradiation with NIR laser for 10 min (**Figure 5d**). These results confirmed the good drug delivery and photoresponsible release ability of Au@Cu_{2-x}S

and superb therapy efficiency of the chemo-photothermal dual-modal synergetic therapy. Additionally, the singlet oxygen production capability of Au@Cu_{2-x}S was evaluated by the 1, 3-diphenylisobenzofuran (DPBF) chemical probe and the fluorescent singlet oxygen sensor green (SOSG) chemical probe (**Figure S12**). The results indicated that the Au@Cu_{2-x}S had the ability to slightly produce ¹O₂ for potential PDT treatment, which might be caused by the copper deficiency [41]. Meanwhile, the cytotoxicity caused by released copper ions before and after NIR irradiation was negligible (**Figure S13**).

Figure 5. a) MTT analysis of MCF-7 and A549 cells incubated with Au@Cu_{2-x}S-PEG at different concentration (10, 25, 50, 100 and 200 µg/mL); b) MTT and c) Calcein-AM/PI staining analysis of MCF-7 cells incubated with DOX (20 µg/mL), Au@Cu_{2-x}S-PEG (100 µg/mL Au@Cu_{2-x}S) and Au@Cu_{2-x}S/DOX-PEG (100 µg/mL Au@Cu_{2-x}S loaded with 20 µg/mL DOX) with or without 980 nm laser irradiation. The cells were co-stained by calcein-AM (live: green) and PI (dead: red). Scale bars: 40 µm. d) CLSM images of MCF-7 MCTSs incubated with DOX, Au@Cu_{2-x}S-PEG and Au@Cu_{2-x}S/DOX-PEG by 980 nm (0.72 W/cm²) laser irradiation for 10 min. Scale bar: 200 µm.

3.6 In Vivo Chemo-Photothermal Dual-Modal Therapy

Using BALB/c tumor-bearing nude mice as model, the in vivo photothermal effect and chemo-photothermal dual-modal anti-cancer capability of Au@Cu_{2-x}S/DOX-PEG were investigated. Compared to slight temperature increase of the PBS-injected control group, both of Au@Cu_{2-x}S-PEG-injected and Au@Cu_{2-x}S/DOX-PEG-injected groups displayed dramatic temperature increase in tumor regions under irradiation by 980 nm laser (**Figure 6a**). The temperature of the tumor regions promptly increased to 63 °C in 5 min irradiation for Au@Cu_{2-x}S-PEG and Au@Cu_{2-x}S/DOX-PEG-treated mice, which was 43 °C higher than that of control group (**Figure S14**). It was attributed to the strong absorption of Au@Cu_{2-x}S at 980 nm and high PT conversion efficiency, providing implication for tumor PT imaging and PTT. In order to investigate the blood circulation behavior of Au@Cu_{2-x}S/-PEG, blood samples were collected intermittently at indicated time points from the mice injected with Au@Cu_{2-x}S/-PEG. Cu₂₊ levels in the blood samples were measured using inductively coupled plasma mass spectrometry (ICP-MS). As shown in the blood circulation curve (**Figure 6b**), it presented a two-compartment model with a first- and second- phase blood circulation time of 0.24 ± 0.14 h and 4.07 ± 0.56 h, respectively, and the relatively long circulation time in the blood was favorable for effective tumor accumulation. To evaluate the in vivo biodistribution, Au@Cu_{2-x}S/-PEG were administered to mice bearing A549 tumors via intravenous injection, followed by measuring of Cu²⁺ in various tissue at 24 h postinjection. Au@Cu_{2-x}S/-PEG were mainly accumulated in the liver and kidney, which were reticuloendothelial system (RES) response for the clearance of foreign nanoparticles. **Figure 6d** and **f** revealed that the Au@Cu_{2-x}S/-PEG mediated PTT treatment delayed the tumor growth compared to the control group. Notably, the chemo-photothermal

dual-modal therapy resulted in remarkable repression of tumors, which were completely eliminated after 15 days, indicating excellent synergetic therapeutic efficacy. The body weight of the mice remained stable during these treatments (**Figure 6e**), suggesting negligible side effects were caused during therapy.

Figure 6. a) Photothermal images of BALB/c tumor-bearing nude mice after injection with PBS, Au@Cu_{2-x}S-PEG and Au@Cu_{2-x}S/DOX-PEG by irradiation with 980 nm laser for different time. b) Blood circulation and c) biodistribution of Au@Cu_{2-x}S-PEG after intravenous injection into A549 tumor-bearing mice, as determined by measuring Cu²⁺ concentration in tissue lysates with ICP-MS; d) Relative tumor growth curves and e) body weight of mice experienced different treatments; f) Photographs of representative mice experienced different treatments.

To provide a deep insight into the mechanism of tumor-growth inhibition and examine the therapeutic efficacy of Au@Cu_{2-x}S/DOX-PEG *in vivo*, the mice were sacrificed 21 days later after therapy and histology analysis of tumor tissues was performed via a typical hematoxylin and eosin (H&E) method. As shown in **Figure 7a**, H&E staining of tumor slices indicated that the tumor cells were seriously damaged as evidenced by severe cell necrosis and apoptosis after the combined

chemo and thermal therapy. Whereas, slight damage was observed in the group with single PTT treatment and negligible damage was found in the control group. Terminal deoxynucleotidyl transferase UTP (Uridine triphosphate) nick end labeling (TUNEL) (**Figure 7b**) staining further confirmed that numerous apoptotic cells in the tumor tissue of the Au@Cu₂-xS/DOX-PEG treated group. The proliferative activity of tumor cells was analyzed by immunostaining against ki-67 (**Figure 7c**). It was obvious that numerous ki-67 positive cells were existed in tumor tissue of the control group. A significant decrease of ki-67 positive cells was detected in the Au@Cu₂-xS-PEG injected group due to the single PTT. Importantly, the Au@Cu₂-xS/DOX-PEG injected group displayed negligible ki-67 positive cells. The histology analysis highlighted the excellent therapeutic efficiency of the chemo-photothermal dual-modal therapy.

Figure 7. a) Hematoxylin and eosin (H&E)-stained slices of tumor tissues collected from different groups after treatment; b) TUNEL staining of tumor tissues collected from different groups after treatment; c) Ki-67 analysis of tumor tissues collected from different groups after treatment.

4. Conclusions

In summary, the LSPR coupling effect between Au core and neighboring semiconductor Cu_{2-x}S shell of rattle-type hollow mesoporous $\text{Au}@\text{Cu}_{2-x}\text{S}$ was optimized by controlling the size of Au core to construct a photoabsorber with high PT conversion efficiency. Microscopic and spectroscopic tools including TEM, SEM, XRD, DLS and Uv-vis etc were employed to comprehensively characterize the morphology and component of the $\text{Au}@\text{Cu}_{2-x}\text{S}$ nanostructures. The efficient surface bioconjugation and cavity drug loading capabilities of the hollow mesoporous structures allowed to delivering gene probe for intracellular oncogenic miRNA detection and constructing chemo-photothermal dual-modal therapy platform. Greatly enhanced in vitro and in vivo antitumor therapy efficacies were realized by combining the NIR photoactivation chemo- and photothermal therapy. The rattle-type metal-semiconductor hollow mesoporous nanostructure with efficient LSPR coupling and high cargo loading capability may benefit future design of chemo-photothermal dual-modal or multi-modal nanomedicine.

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Supporting Information

Supplementary data related to this article can be found.

References

- [1] Zou L, Wang H, He B, Zeng L, Tan T, Cao H, et al. Current Approaches of Photothermal Therapy in Treating Cancer Metastasis with Nanotherapeutics. *Theranostics*. 2016;6:762-72.
- [2] Chen G, Roy I, Yang C, Prasad PN. Nanochemistry and Nanomedicine for Nanoparticle-based Diagnostics and Therapy. *Chem Rev*. 2016;116:2826-85.
- [3] Abbas M, Zou Q, Li S, Yan X. Self-Assembled Peptide- and Protein-Based Nanomaterials for Antitumor Photodynamic and Photothermal Therapy. *Adv Mater*. 2017.
- [4] Huang X, Jain PK, El-Sayed IH, El-Sayed MA. Plasmonic photothermal therapy (PPTT) using gold nanoparticles. *Lasers Med Sci*. 2008;23:217-28.
- [5] Chen Q, Wen J, Li H, Xu Y, Liu F, Sun S. Recent advances in different modal imaging-guided photothermal therapy. *Biomaterials*. 2016;106:144-66.
- [6] Riedinger A, Avellini T, Curcio A, Asti M, Xie Y, Tu R, et al. Post-Synthesis Incorporation of ^{64}Cu in CuS Nanocrystals to Radiolabel Photothermal Probes: A Feasible Approach for Clinics. *J Am Chem Soc*. 2015;137:15145-51.
- [7] Wang Z, Huang P, Jacobson O, Wang Z, Liu Y, Lin L, et al. Biomineralization-Inspired Synthesis of Copper Sulfide-Ferritin Nanocages as Cancer Theranostics. *ACS Nano*. 2016;10:3453-60.
- [8] Song J, Wang F, Yang X, Ning B, Harp MG, Culp SH, et al. Gold Nanoparticle Coated Carbon Nanotube Ring with Enhanced Raman Scattering and Photothermal Conversion Property for Theranostic Applications. *J Am Chem Soc*. 2016;138:7005-15.
- [9] Li Q, Hong L, Li H, Liu C. Graphene oxide-fullerene C_{60} (GO- C_{60}) hybrid for photodynamic and photothermal therapy triggered by near-infrared light. *Biosens Bioelectron*. 2017;89:477-82.
- [10] Li L, Chen C, Liu H, Fu C, Tan L, Wang S, et al. Multifunctional Carbon-Silica Nanocapsules with Gold Core for Synergistic Photothermal and Chemo-Cancer Therapy under the Guidance of Bimodal Imaging. *Adv Funct Mater*. 2016;26:4252-61.
- [11] Wang J, Tan X, Pang X, Liu L, Tan F, Li N. MoS₂ Quantum Dot@Polyaniline Inorganic-Organic Nanohybrids for in Vivo Dual-Modal Imaging Guided Synergistic Photothermal/Radiation Therapy. *ACS Appl Mater Interfaces*. 2016;8:24331-8.
- [12] Meng X, Liu Z, Cao Y, Dai W, Zhang K, Dong H, et al. Fabricating Aptamer-Conjugated PEGylated-MoS₂/Cu_{1.8}S Theranostic Nanoplatform for Multiplexed Imaging Diagnosis and Chemo-Photothermal Therapy of Cancer. *Adv Funct Mater*. 2017;27:1605592.
- [13] Sun Z, Xie H, Tang S, Yu X-F, Guo Z, Shao J, et al. Ultrasmall Black Phosphorus Quantum Dots: Synthesis and Use as Photothermal Agents. *Angew Chem Int Ed*. 2015;54:11526-30.
- [14] Chen W, Ouyang J, Liu H, Chen M, Zeng K, Sheng J, et al. Black Phosphorus Nanosheet-Based Drug Delivery System for Synergistic Photodynamic/Photothermal/Chemotherapy of Cancer. *Adv Mater*. 2016;1603864.

- [15] Lin H, Wang X, Yu L, Chen Y, Shi J. Two-Dimensional Ultrathin MXene Ceramic Nanosheets for Photothermal Conversion. *Nano Lett.* 2017;17:384-91.
- [16] Xue Q, Zhang H, Zhu M, Pei Z, Li H, Wang Z, et al. Photoluminescent Ti₃C₂ MXene Quantum Dots for Multicolor Cellular Imaging. *Adv Mater.* 2017;1604847.
- [17] Hsu S-W, On K, Tao AR. Localized Surface Plasmon Resonances of Anisotropic Semiconductor Nanocrystals. *J Am Chem Soc.* 2011;133:19072-5.
- [18] Chen H, Shao L, Li Q, Wang J. Gold nanorods and their plasmonic properties. *Chem Soc Rev.* 2013;42:2679-724.
- [19] Zheng T, Li GG, Zhou F, Wu R, Zhu J-J, Wang H. Gold-Nanosponge-Based Multistimuli-Responsive Drug Vehicles for Targeted Chemo-Photothermal Therapy. *Adv Mater.* 2016;28:8218-26.
- [20] Du Y, Jiang Q, Beziere N, Song L, Zhang Q, Peng D, et al. DNA-Nanostructure-Gold-Nanorod Hybrids for Enhanced In Vivo Optoacoustic Imaging and Photothermal Therapy. *Adv Mater.* 2016.
- [21] Chen W, Zhang S, Yu Y, Zhang H, He Q. Structural-Engineering Rationales of Gold Nanoparticles for Cancer Theranostics. *Adv Mater.* 2016;28:8567-85.
- [22] Ming T, Zhao L, Yang Z, Chen H, Sun L, Wang J, et al. Strong polarization dependence of plasmon-enhanced fluorescence on single gold nanorods. *Nano Lett.* 2009;9:3896-903.
- [23] Li Y, Lu W, Huang Q, Li C, Chen W. Copper sulfide nanoparticles for photothermal ablation of tumor cells. *Nanomedicine : nanotechnology, biology, and medicine.* 2010;5:1161-71.
- [24] Tian Q, Tang M, Sun Y, Zou R, Chen Z, Zhu M, et al. Hydrophilic flower-like CuS superstructures as an efficient 980 nm laser-driven photothermal agent for ablation of cancer cells. *Adv Mater.* 2011;23:3542-7.
- [25] Zhang S, Sun C, Zeng J, Sun Q, Wang G, Wang Y, et al. Ambient Aqueous Synthesis of Ultrasmall PEGylated Cu_{2-x}Se Nanoparticles as a Multifunctional Theranostic Agent for Multimodal Imaging Guided Photothermal Therapy of Cancer. *Adv Mater.* 2016;28:8927-36.
- [26] Bi H, Dai Y, Lv R, Zhong C, He F, Gai S, et al. Doxorubicin-conjugated CuS nanoparticles for efficient synergistic therapy triggered by near-infrared light. *Dalton Trans.* 2016;45:5101-10.
- [27] Ding X, Liow CH, Zhang M, Huang R, Li C, Shen H, et al. Surface Plasmon Resonance Enhanced Light Absorption and Photothermal Therapy in the Second Near-Infrared Window. *J Am Chem Soc.* 2014;136:15684-93.
- [28] Ji M, Xu M, Zhang W, Yang Z, Huang L, Liu J, et al. Structurally Well-Defined Au@Cu_{2-x}S Core-Shell Nanocrystals for Improved Cancer Treatment Based on Enhanced Photothermal Efficiency. *Adv Mater.* 2016;28:3094-101.
- [29] Dong H, Lei J, Ding L, Wen Y, Ju H, Zhang X. MicroRNA: function, detection, and bioanalysis. *Chem Rev.* 2013;113:6207-33.
- [30] Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science.* 2004;303:83-6.
- [31] Li L, Feng J, Liu H, Li Q, Tong L, Tang B. Two-color imaging of microRNA with enzyme-free signal amplification via hybridization chain reactions in living cells. *Chem Sci.* 2016;7:1940-5.
- [32] Ma W, Fu P, Sun M, Xu L, Kuang H, Xu C. Dual Quantification of MicroRNAs and Telomerase in Living Cells. *J Am Chem Soc.* 2017;139:11752-9.
- [33] Cheglakov Z, Cronin TM, He C, Weizmann Y. Live Cell MicroRNA Imaging Using Cascade Hybridization Reaction. *J Am Chem Soc.* 2015;137:6116-9.
- [34] Bastus NG, Comenge J, Puntès V. Kinetically controlled seeded growth synthesis of

- citrate-stabilized gold nanoparticles of up to 200 nm: size focusing versus Ostwald ripening. *Langmuir*. 2011;27:11098-105.
- [35] Ramadan S, Guo L, Li Y, Yan B, Lu W. Hollow copper sulfide nanoparticle-mediated transdermal drug delivery. *Small*. 2012;8:3143-50.
- [36] Song G, Li C, Hu J, Zou R, Xu K, Han L, et al. A simple transformation from silica core-shell-shell to yolk-shell nanostructures: a useful platform for effective cell imaging and drug delivery. *J Mater Chem*. 2012;22:17011.
- [37] Hu J, Song G, Han L, Zou W, Xiao Z, Huang X, et al. A Novel Photothermal Nanocrystals of Cu₇S₄ Hollow Structure for Efficient Ablation of Cancer Cells. *Nano-Micro Letters*. 2014;6:169.
- [38] Zhu H, Wang J, Wu D. Fast synthesis, formation mechanism, and control of shell thickness of CuS hollow spheres. *Inorg Chem*. 2009;48:7099-104.
- [39] Zhang H, Zhu Q, Zhang Y, Wang Y, Zhao L, Yu B. One-Pot Synthesis and Hierarchical Assembly of Hollow Cu₂O Microspheres with Nanocrystals-Composed Porous Multishell and Their Gas-Sensing Properties. *Adv Funct Mater*. 2007;17:2766-71.
- [40] Xu H, Wang W. Template synthesis of multishelled Cu₂O hollow spheres with a single-crystalline shell wall. *Angew Chem Int Ed*. 2007;46:1489-92.
- [41] Wang S, Riedinger A, Li H, Fu C, Liu H, Li L, et al. Plasmonic copper sulfide nanocrystals exhibiting near-infrared photothermal and photodynamic therapeutic effects. *ACS Nano*. 2015;9:1788-800.
- [42] Hessel CM, Pattani VP, Rasch M, Panthani MG, Koo B, Tunnell JW, et al. Copper selenide nanocrystals for photothermal therapy. *Nano Lett*. 2011;11:2560-6.
- [43] Tian Q, Jiang F, Zou R, Liu Q, Chen Z, Zhu M, et al. Hydrophilic Cu₉S₅ nanocrystals: a photothermal agent with a 25.7% heat conversion efficiency for photothermal ablation of cancer cells in vivo. *ACS Nano*. 2011;5:9761-71.
- [44] Zeng J, Goldfeld D, Xia Y. A plasmon-assisted optofluidic (PAOF) system for measuring the photothermal conversion efficiencies of gold nanostructures and controlling an electrical switch. *Angew Chem Int Ed*. 2013;52:4169-73.
- [45] Oskooi AF, Roundy D, Ibanescu M, Bermel P, Joannopoulos JD, Johnson SG. Meep: A flexible free-software package for electromagnetic simulations by the FDTD method. *Comput Phys Commun*. 2010;181:687-702.
- [46] Farjadpour A, Roundy D, Rodriguez A, Ibanescu M, Bermel P, Joannopoulos JD, et al. Improving accuracy by subpixel smoothing in the finite-difference time domain. *Opt Lett*. 2006;31:2972.
- [47] Barnes DG, Vidiassov M, Ruthensteiner B, Fluke CJ, Quayle MR, McHenry CR. Embedding and publishing interactive, 3-dimensional, scientific figures in Portable Document Format (PDF) files. *PLoS One*. 2013;8:e69446.
- [48] Johnson PB, Christy RW. Optical Constants of the Noble Metals. *Phys Rev B*. 1972;6:4370-9.
- [49] Deng X, Li K, Cai X, Liu B, Wei Y, Deng K, et al. A Hollow-Structured CuS@Cu₂S@Au Nanohybrid: Synergistically Enhanced Photothermal Efficiency and Photoswitchable Targeting Effect for Cancer Theranostics. *Adv Mater*. 2017;1701266.