Electronic Supplementary Information for:

Design, synthesis, and characterization of Ag-Bi-S-based multifunctional nanotheranostic platform

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1. Experimental Section

1.1. Materials

1-Octanethiol (≥98.5%), poly(acrylic acid) (PAA, average Mn = 1800), bismuth (III) neodecanoate, oleic acid (OAc), and n-octylamine were purchased from Sigma-Aldrich. 1-Octadecene (ODE, tech. 90%) was purchased from ACROS. Zinc acetate ($Zn(Ac)_2$), oleylamine (OAm), N,N-dimethylformamide (DMF), and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) were purchased from Aladdin. Triethylamine, silver acetate (AgAc), S powder, Bi_2O_3, dimethyl sulfoxide (DMSO), chloroform, methanol, and ethanol were purchased from Sinopharm Group Co. Ltd. Methoxy poly(ethylene glycol) terminated with $-NH_2$ ($mPEG-NH_2$, Mn = 2000) were purchased from ShangHai ToYongBio. Tech. Inc.

1.2. Synthesis of Ag-Bi-S nanocrystals with different structure

1.2.1. Preparation of precursor solutions

S precursor solution was prepared by dissolving 4.0 mg of S powder in 7.5 mL of ODE under ultrasound.

Bismuth (III) neodecanoate was mixed with a certain amount of ODE to prepare Neo-Bi precursor solution of different concentration (1 M, 0.8 M, and 0.16 M).

1.0 mL of bismuth (III) neodecanoate was mixed with 0.6 mL of OAm to prepare OAm-Bi precursor solution (1 M).

OAc-Bi precursor solution (1 M) was prepared as follows: 2.33 g of Bi_2O_3 was added into 10 mL of acetate under Ar atmosphere. During the heating process, the colour of solid changed from faint yellow to white. The temperature of reaction was maintained at 120°C until all the liquid has evaporated. Then 9.5 mL of OAc was added into the solid at 130°C. The reaction stopped after 5 h to obtain a transparent solution.

Zn:Bi precursor was prepared by mixing 370 mg of $Zn(Ac)_2$ in 10 mL of OAm/ODE (1:4, v/v) and 0.5 mL of Neo-Bi precursor solution (0.8 M).

1.2.2. Synthesis of Ag_2S:Bi nanocrystals.

5 mL of ODE, 16.7 mg of AgAc, 315 μL of Neo-Bi precursor (0.16 M), and 260 μL of 1-octanethiol were loaded into a three-neck flask filled with Ar gas. With the increase of temperature, the mixture changed from yellow emulsion to yellow transparent solution. When the temperature increased to 160°C, 1.5 mL of S precursor was rapidly injected, and the subsequent nanocrystals growth proceeded at 130°C for 30 min. Finally, the products were purified by precipitate with methanol/ethanol (2:3, v/v).

1.2.3. Synthesis of Ag_2S:Bi/ZnS:Bi nanocrystals.

Firstly, Ag_2S:Bi nanocrystals were prepared with Ag:Bi feed ratio of 5:1. Without any purification, the Ag_2S:Bi nanocrystals were directly coated with ZnS:Bi shell by dropwise adding 1 mL of Zn:Bi precursor.

1.2.4. Synthesis of Ag_2S/Bi_2S_3 nanocrystals.

Firstly, Ag_2S nanocrystals were prepared without the addition of Bi precursor. Then 1 mL of OAm-Bi, OAc-Bi or Neo-Bi precursor (1 M) was dropwise added or injected into the reaction mixture followed by 30 min growth.

1.3. Water-soluble modification of Ag_2S/Bi_2S_3 nanocrystals

Amphiphilic C8-PAA-PEG polymer was applied for water-soluble modification of Ag_2S/Bi_2S_3 nanocrystals. Firstly, C8-PAA-PEG was prepared *via* n-octylamine and mPEG-NH_2 grafting on PAA. Typically, 0.1 mmol of PAA, 2.25 mmol of EDC·HCl, and 0.8 mmol of n-octylamine were mixed in 8 mL of DMF and stirred for 24 h at room temperature. Then, 0.3 mmol of EDC·HCl and 0.2 mmol of mPEG-NH_2 were added, and the reaction proceeded for another 24 h. After purified by dialysis, the product was lyophilized for further surface modification. Then, hydrophobic Ag_2S/Bi_2S_3 nanocrystals and C8-PAA-PEG were fully mixed in chloroform at the mass ratio of 1:5. After chloroform was evaporated completely, the residue was dispersed in 1×PBS to obtain hydrophilic Ag_2S/Bi_2S_3 nanocrystals, denoted as Ag_2S/Bi_2S_3-PEG nanocrystals.

1.4. Characterization

Transmission electron microscopy (TEM) images were acquired on a JEOL JEM-2100 transmission electron microscope with an acceleration voltage of 200 kV, which was equipped with an energy-dispersive X-ray (EDX) spectrometer (EDAX Inc.) to conduct the EDX element analysis. Fluorescence (FL) emission spectra were measured on a Fluorolog-3 FL spectrophotometer (HORIBA Jobin Yvon Inc.) equipped with an InGaAs detector. Inductively coupled plasma-optical emission spectrometry (ICP-OES) analyses were conducted on a PerkinElmer Avio 200 spectrometer. Powder X-ray diffraction (XRD) analysis was conducted on a Bruka D8 Advanced X-Ray diffractometer. X-ray photoelectron spectroscopy (XPS) spectra were collected on a Thermofisher Scientific EscaLabXi+ X-ray photoelectron spectrometer. Dynamic light scattering (DLS) measurements were performed on a Malvern Zetasizer Ultra instrument. CT images were collected using a GMI Triumph X-SPECT/X-OCT imaging system. The photothermal conversion performance was evaluated by irradiating the sample solutions with a MDL-III-808 laser (1.0 W/cm^2) and recording the temperature change using a Magnity MY320 infrared thermal imaging camera.

1.5. Determination of FL quantum yield

Ag_2S/Bi_2S_3-PEG nanocrystals and ICG were individually dispersed in water and DMSO, and subsequently diluted to five different concentrations with the absorbance at 650 nm ranging from 0.05 to 0.01. Under identical conditions, the FL emission spectra of these five solutions with different concentrations were measured with the excitation wavelength of 650 nm. Then, the integrated FL intensity was plotted against the absorbance, and the quantum yield of Ag_2S/Bi_2S_3-PEG nanocrystals was calculated using the following equation,

$$\phi_{Sample} = \phi_{ICG}\left(\frac{Grad_{Sample}}{Grad_{ICG}}\right)\left(\frac{\eta_{water}}{\eta_{DMSO}}\right)^2$$

where Φ is the quantum yield, *Grad* is the gradient from the plot of integrated FL intensity *vs.* absorbance, and η is the refractive indexes of solvent.

1.6. Cell culture and CCK-8 assay

HeLa cells were cultured in DMEM (Gibco) containing 10% (v/v) FBS (Gibco) and 1% (w/v) penicillin/streptomycin (Gibco) at 37ºC in a 5% CO_2 atmosphere.

For CCK-8 assay, HeLa cells resuspended in DMEM culture medium were seeded into 96-well plates at a density of 5000 cells per well and cultured overnight for cell attachment. Then the medium was replaced with 100 μL of fresh medium containing different concentration of Ag_2S/Bi_2S_3-PEG nanocrystals (0, 5, 10, 20, 50, 100, 200, 400 μg/mL). After incubation for 24 h, the cells in each well were washed once with 1×PBS and incubated with CCK-8 diluted with DMEM medium (1:9, v/v) for another 2 h. Then the absorbance of each well at 450 nm was recorded using a microplate spectrophotometer (MULTISKAN MK3, Thermo Scientific).

1.7. Calcein-AM/PI cell staining

HeLa cells were seeded at a density of 6×10^5 cells per dish into confocal petri dishes. After incubation overnight, the medium was replaced with 2 mL of fresh medium containing Ag_2S/Bi_2S_3-

PEG nanocrystals (100 μg/mL) and further incubated for 12 h. Subsequently, the cells were exposed to the 808 nm laser at a power density of 1.0 W/cm^2 for 5 min and 10 min. Meanwhile, HeLa cells without the treatment of Ag_2S/Bi_2S_3-PEG nanocrystals and with only laser irradiation were set as the control group. Finally, Calcein AM (3 μM) was added to each dish and incubated for 30 min, followed by 5-min incubation with PI (4 μM) to obtain red (dead) and green (live) FL images of HeLa cells.

2. Supporting Figures

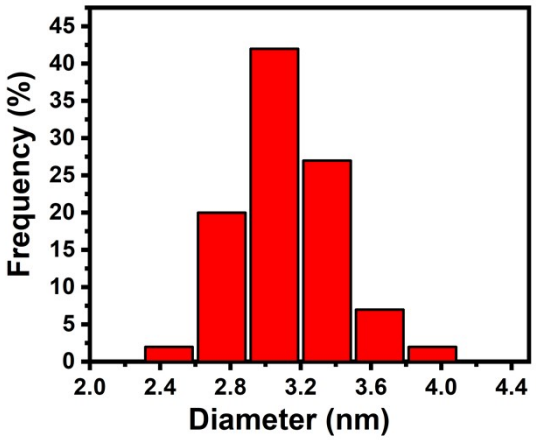

Fig. S1 Size histogram of Ag_2S/Bi_2S_3 nanocrystals.

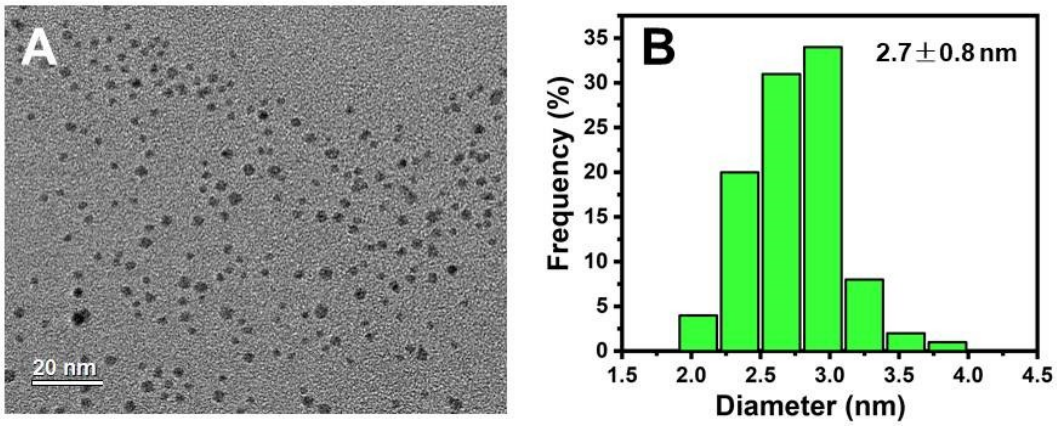

Fig. S2 (A) TEM image and (B) size histogram of Ag_2S nanocrystals.

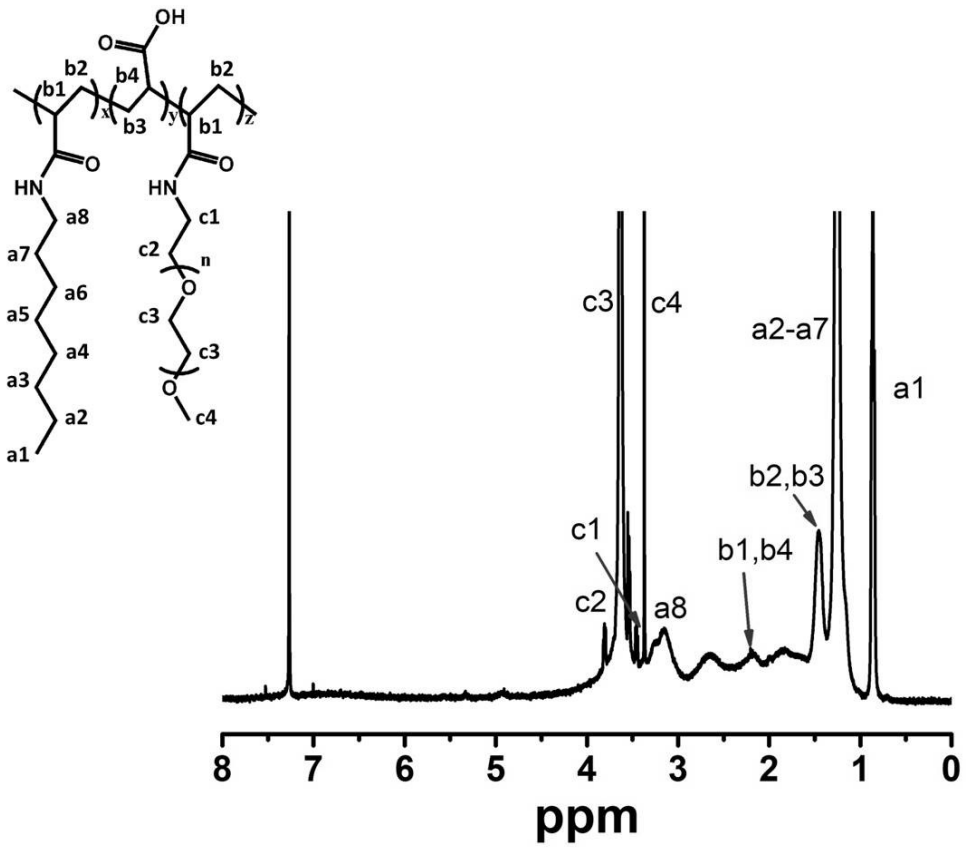

Fig. S3 ^{1}H NMR spectrum of C8-PAA-PEG polymer.

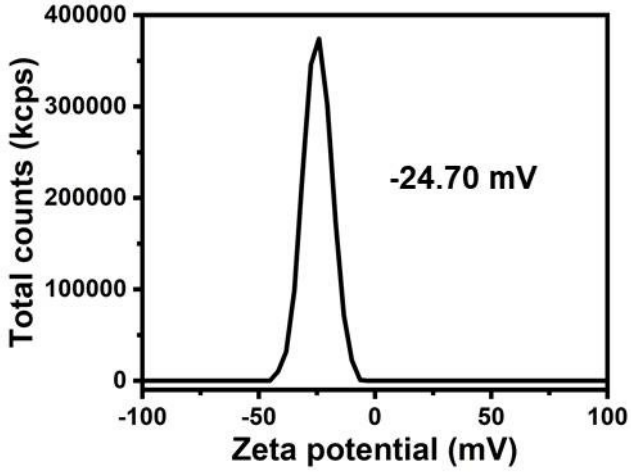

Fig. S4 Zeta potential of hydrophilic Ag_2S/Bi_2S_3-PEG nanocrystals.

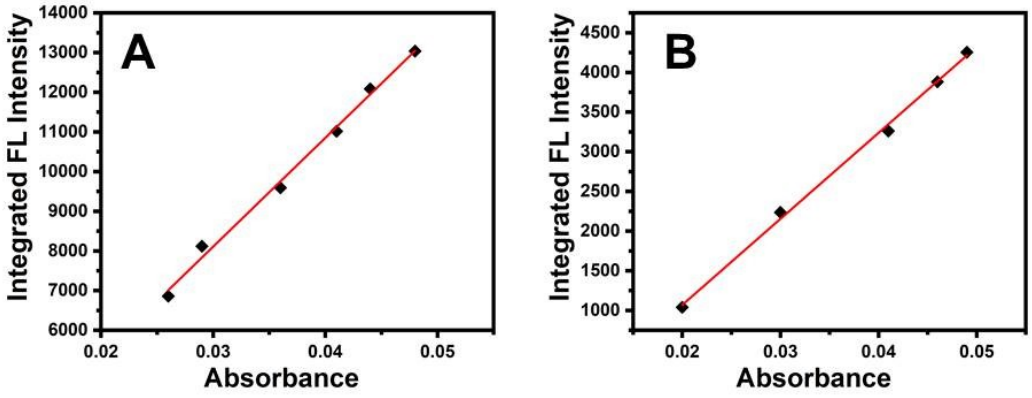

Fig. S5 Integrated FL intensity *vs.* absorbance of ICG in DMSO (A) and Ag_2S/Bi_2S_3-PEG nanocrystals in water (B).

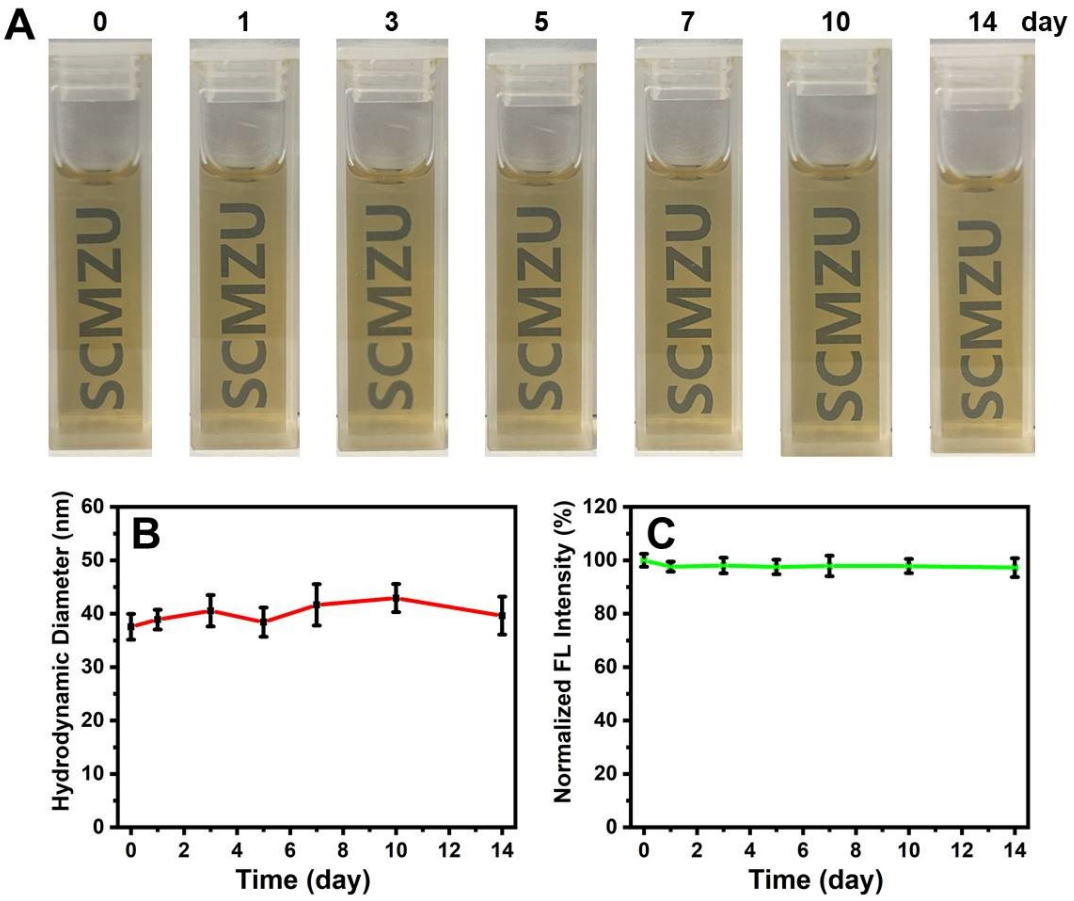

Fig. S6 Pictures (A), hydrodynamic diameter change curve (B), and FL intensity change curve (C) of Ag_2S/Bi_2S_3-PEG nanocrystals during the storage in 1×PBS at 4°C for 14 days.

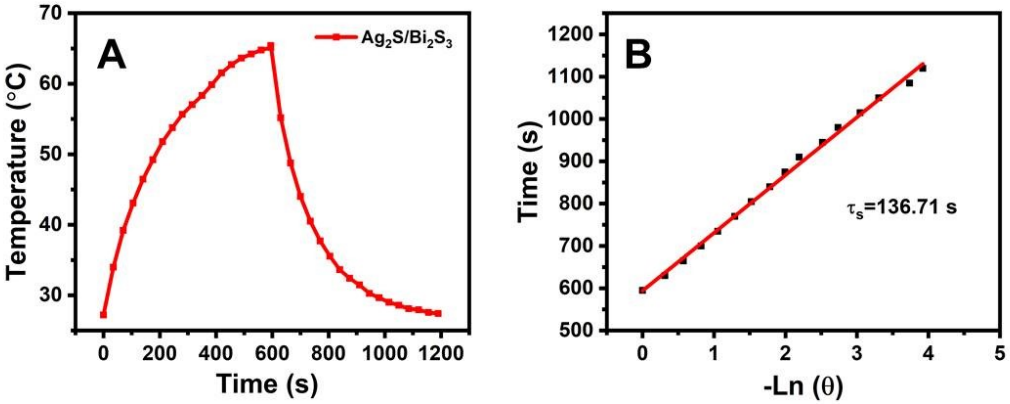

Fig. S7 (A) Temperature elevation profiles of Ag_2S/Bi_2S_3 nanocrystals upon 808 nm laser irradiation (1.0 W/cm^2, 6 min) and then the laser was shut off. (B) Plot of the cooling time versus -Ln(θ) obtained from the cooling stage as shown in (A).