Supplementary Information-

Polyphenol-assisted Albumin-based Biomineralization Nanocarriers with NIR-II Targeted Photothermal Performance towards Broad-spectrum Radicals Scavenging

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Materials

Copper nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$, 99.99%), tannic acid (TA, $C_{76}H_{52}O_{46}$, ACS), bovine serum albumin (BSA, ≥98.0%), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH, contains 10-20% benzene), iron sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, AR) and salicylic acid (SA) were all purchased from Aladdin chemistry Co. Ltd. Ethyl alcohol (C_2H_5OH, AR) , sodium hydroxide (NaOH, AR) and hydrogen peroxide (H_2O_2, 30 %) were supplied by Chengdu Kelong Inc. Deionized (D.I.) water was homemade and used during the whole study. All the chemicals were used without any further purification.

Characterization

Scanning electron microscopy (SEM, Apreo S Hivoc, Thermo Fisher Scientific, FEI) and transmission electron microscopy (TEM, Talors F200i, Thermo Scientific) were used to observe the surface morphology of TA-BSA@CuS. The hydrodynamic sizes and zeta potentials of TA-BSA@CuS were detected by the zeta-sizer (Nano ZS, Marvin, UK). Powder X-ray diffraction (XRD, Ultima IV, Rigaku, Japan) analyses were conducted to characterize the TA-BSA@CuS. Raman spectrum characterization was carried out on a raman spectroscopy (inVia Reflex, Renishaw, U.S.A.). The chemical compositions of TA-BSA@CuS were characterized by X-ray photoelectron spectroscopy (XPS spectra, XSAM800, Kratos Analytical, U.K.) and Fourier transform infrared spectroscopy (FTIR, Thermo Scientific iS50, U.S.A.) with the wavenumber ranging from 500 to 4000 cm^{-1}. A UV-vis spectrophotometer (UV-6100S, MAPADA, China) was employed to investigate the UV-visible adsorption ability of the samples. Flow cytometry (cytoflex, Beckman, China) was performed with excitation and emission at 488 nm and 525 nm respectively to determine intracellular ROS.

Photothermal Activities of TA-BSA@CuS

To evaluate the photothermal effects of TA-BSA@CuS, the photothermal conversion ability of the TA-BSA@CuS under a near infrared (NIR, KA64HAMFA, BWT) laser was measured by an infrared camera (Fluke TiS60+, U.S.A). TA-BSA@CuS aqueous solution with different concentrations (0, 10, 20, 30, 40, 50 μg/mL) were exposed to the 808 nm or 1064 nm laser with a uniform distance for 5 min. Meanwhile, a thermal imaging camera was utilized to take images every 10 seconds (s). To evaluate the photostability, a solution of TA-BSA@CuS (50 μg/mL) was

irradiated with 808 nm and 1064 nm laser (1.0 W/cm^2) for 5 min (Laser On), followed by 5 min without irradiation (Laser Off). An addition five laser On/Off cycles were then repeated to test the photostability. In addition, a pork or pig skin was pasted on the surface of the container as a barrier layer to study the effect of biological tissue on the photothermal conversion of the materials, which was irradiated with 808 nm and 1064 nm laser (1.0 W/cm^2) for 5 min.

Biocompatibility of TA-BSA@CuS *in vitro*

Hemolysis Ratio

The hemolysis was aimed to evaluate the compatibility of red blood cells (RBCs) and TA-BSA@CuS. Firstly, 2 mL of the whole blood was added to 8 mL of phosphate buffered saline (PBS) without calcium and magnesium, then RBCs was isolated from plasma by centrifuging at 2000 rpm for 10 min, the centrifugation procedure was repeated at least 5 times until the supernatant and the interface were clear. For the hemolysis test, diluted RBCs (0.2 mL, approx.10^8 cells per mL) were mixed with PBS solution (0.8 ml, pH=7.4) as the negative control, and the D.I. water (0.8 mL) is added into the diluted RBCs solution as the positive control. TA-BSA@CuS or BSA@CuS were severally dispersed in 0.8 mL of PBS (pH=7.4) which were previously immersed in PBS overnight as experimental samples. Then the diluted RBCs added to sample and incubated at 37 °C for 3 hours (h). The prepared solutions were divided into NIR (+) and NIR (-) group. The NIR (+) groups were irradiated by NIR (1064 nm, 1.0 W/cm^2) for 5 min, and the NIR (-) groups were incubated without NIR. Afterwards, the mixtures were centrifuged at 7000 rpm for 5 min, and the absorbances of the released hemoglobin in the suspensions were measured by microplate reader (Multiskan SkyHigh, Thermo Scientific) at 540 nm and then the hemolysis ratios could be calculated by the following formula:

$$\text{Hemolysis ratio (\%)} = (\text{As}-\text{An})/(\text{Ap}-\text{An})\times 100\% \quad (1)$$

where As is the absorbance of the suspensions in the presence of TA-BSA@CuS, Ap and An are the absorbance of positive control and the negative control, respectively.

Complement Activation and Contact Activation

Enzyme-linked immune sorbent assay (ELISA) was used to evaluate platelet activation (Thermo Fisher Human PF4 ELISA Kit), coagulation activation (AssayMax™ Human TAT Complex ELISA Kit), and complement activation (BD OptEIATM Human C3a ELISA Kit). The TA-BSA@CuS were

pre-incubated in PBS in a 24-well cell culture plate overnight, and 50 μg/mL of TA-BSA@CuS in each group were incubated with 250 μL of fresh blood (anticoagulated with 16000 ATU/mL recombinant hirudin, anticoagulant-to-blood ratio = 1:39 (v/v)). After being incubated at 37 ℃ for 1 h, the whole blood was centrifuged at 6500 rpm for 15 min, and the supernatant was diluted 1/40000 for the C3a test. 100 μL of the diluted sample or standard solution was added to the ELISA test well plate coated with specific antibody. For the PF4 test, the supernatant was diluted 1/400, 200 μL of the tested samples or standard solutions were introduced into the corresponding ELISA plate well and the plate was incubated at 20 °C for 1 h. Finally, the detections were carried out according to the respective instruction manuals.

For the TAT test, fresh whole blood anticoagulated with sodium citrate (anticoagulant-to-blood = 1:9 (v/v)) was collected, and the whole blood was centrifuged at 4000 rpm for 15 min to obtain PPP. Then, 50 μg/mL of TA-BSA@CuS in each group were incubated with 150 μL PPP, and 10 μL of $CaCl_2$ solution was added to reactivate the coagulation cascade. The nanoparticles were incubated with re-calcified PPP at 37 °C for 30 min. The generated concentrations of TAT were determined by a commercial ELISA kit. Thirdly, 50 μL of the sample was added in the 96-well test plate and the plate was incubated at 20 °C for 2 h. Finally, the detections were carried out according to the respective instruction manuals.

And the concentrations of C3a, PF4 and TAT were calculated with reference to the standard curve. The primary blood without any treatment was used as blank control.

Blood Routine

For blood routine tests, the TA-BSA@CuS were pre-incubated in PBS overnight. And 50 μg/mL of TA-BSA@CuS in each group were incubated with 200 μL of fresh blood (anticoagulated with 1.5 g/L EDTA-K2) at 37 °C for 1.5 h. The blood co-incubated with the hydrogel spheres was collected and tested by a hematology analyzer (Mindray BC-5100, China).

Cytotoxicity Study

Human renal proximal tubular epithelial cells (HK-2) (Shanghai Zhongqiao Xinzhou Biotechnology Co. Ltd) were cultured in Dulbecco's Modified Eagle Medium/F-12 (DMEM/F12) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin in a humidified incubator containing 5% CO_2 at 37 °C according to American Type Culture Collection suggestions.

HK-2 cells were seeded into a 12-well plate with a concentration of 5×10^5 cell/mL, and cultured in an incubator until cells were completely adherent. Then culture medium in each well was replaced by fresh DMEM containing TA-BSA@CuS at different concentrations. The prepared solutions were divided into NIR (+) and NIR (-) groups. The NIR (+) group was irradiated by NIR (1064 nm, 1.0 W/cm^2) for 10 min, and the NIR (-) group was incubated without NIR. The cytotoxicity was assessed after 24-hour incubation by the ATP assay. According to the manufacturer's instructions of ATP assay kit (S0026, Beyotime).

$ABTS^{\bullet+}$ Scavenging Activity

The solution containing $ABTS^{\bullet+}$ is prepared according to the work of Wei *et al.* [1] $ABTS^{\bullet+}$ solution was added to TA-BSA@CuS. The prepared solutions were divided into NIR (+) and NIR (-) groups. The NIR (+) group was irradiated with 808 nm or 1064 nm (1.0 W/cm^2) for 5 min, and the NIR (-) group was incubated without NIR. Afterward, the supernatant was collected by centrifugation (12000 rpm, 5 min) to remove the nanoparticle and the absorbance was measured at 734 nm. The scavenging capability of $ABTS^{\bullet+}$ was calculated using the following equation:

$$ABTS^{\bullet+} \text{ scavenging effect (\%)} = (Ac-As)/Ac\times100\% \quad (3)$$

where, Ac is the initial concentration of the $ABTS^{\bullet+}$ and As is absorbance of the remaining concentration of $ABTS^{\bullet+}$ in the presence of TA-BSA@CuS.

DPPH Free Radical Scavenging Activity

DPPH scavenging assay was used to evaluate the antioxidative activity of TA-BSA@CuS. Briefly, the TA-BSA@CuS (20 μg/mL, 500 μL) was mixed with DPPH ethanol solution (0.4 mM, 500 μL). The prepared TA-BSA@CuS/DPPH mixed solution were divided into NIR (+) and NIR (-) groups. The NIR (+) group was irradiated by NIR with 808 nm or 1064 nm (1.0 W/cm^2) for 5 min, and the NIR (-) group was incubated without NIR. Then the absorbance was measured at 517 nm, and the DPPH radical scavenging capacity of TA-BSA@CuS was calculated using Equation

$$\text{DPPH scavenging ratio (\%)} = (Ac-As)/Ac\times100\% \quad (2)$$

where Ac is the absorbance of the blank control which only contains DPPH solution and As is the absorbance of DPPH solution in the presence of TA-BSA@CuS.

Catalase (CAT) Activity

In order to evaluate the H_2O_2 scavenging capacity, we prepare two solutions A and B. Solution A

of H_2O_2 (10 mM) was prepared in PBS, solution B was prepared by mixing $TiSO_4$ aqueous solution (20 mM) and H_2SO_4 (98%, 8.3 mL) to H_2O (50 mL). Then, solution A (500 μL) was added to TA-BSA@CuS solution (100 μg/mL, 500 μL). The prepared solutions were divided into NIR (+) and NIR (-) groups. The NIR (+) group was irradiated with 808 nm and 1064 nm (1.0 W/cm^2) for 15 min, and the NIR (-) group was incubated without NIR. Then, the supernatant was collected by centrifugation (12000 rpm, 5 min) to remove the nanoparticle. Add supernatant (50 μL) to solution B (100 μL) and measure the absorbance at 405 nm. The solution containing H_2O_2 without TA-BSA@CuS was defined as the control. The formula percentage of H_2O_2 scavenging of TA-Cu@BSA was calculated using the following equation:

$$H_2O_2 \text{ scavenging effect } (\%) = (Ac-As)/Ac \times 100\% \quad (4)$$

where, Ac is the absorbance of the control, and As is the absorbance in the presence of TA-BSA@CuS.

In addition, the O_2 generation was measured to assess the CAT activity of TA-BSA@CuS. The TA-BSA@CuS was added into 0.1 M H_2O_2 PBS solution at room temperature, and the prepared solutions (25 μg/mL) were divided into NIR (+) and NIR (-) groups. The NIR (+) group was irradiated by NIR (1064 nm, 1.0 W/cm^2), and the NIR (-) group was stored in the dark environment. Meanwhile, the O_2 concentration was measured by dissolved oxygen meter (METTLER TOLEDO, S9-Field Kit). To directly observe O_2 bubbles, we transferred the reaction into a small container and recorded it with a camera.

Hydroxyl Radical (•OH) Scavenging Activity

Hydroxyl radical (•OH) scavenging efficiency of TA-BSA@CuS with different concentrations was achieved by measuring the presence of the specific probe Salicylic Acid (SA). An obvious absorption peak at 510 nm was observed after mixing SA with the Fe^{2+}/H_2O_2 system. Fe^{2+} solution (450 μL) was added to H_2O_2 solution (450 μL), and then the TA-BSA@CuS of various concentrations was added to mixed solution. The prepared solutions were divided into NIR (+) and NIR (-) groups. The NIR (+) group was irradiated by NIR (1064 nm, 1.0 W/cm^2) for 10 min, and the NIR (-) group was incubated without NIR. Then, the supernatant was collected by centrifugation, then the absorbance was measured at 510 nm. The percentage of •OH scavenging efficiency was calculated by using the formula given bellow:

$$\bullet OH \text{ scavenging effect } (\%) = (Ac\text{-}As)/Ac \times 100\% \quad (5)$$

where, Ac is the absorbance of the control, and As is the absorbance in the presence of TA-BSA@CuS.

Antibacterial Activity *in vitro*

In this study, *Escherichia coli* (*E. coli*, ATCC25922) and *Staphylococcus aureus* (*S. aureus,* ATCC 6538) were chosen as the model of Gram-negative and Gram-positive bacteria, respectively. We determined the number of bacteria by the value of OD600. The bacteria suspensions used throughout the antibacterial activity were 10^5 CFU/mL for *E. coli* and 10^5 CFU/mL for *S. aureus*. The Mueller-Hinton Agar (MHA) and Mueller-Hinton Broth (MHB) were used as culture media.

The antibacterial activity of TA-BSA@CuS against *E. coli* and *S. aureus* were tested by agar plate counting method and SEM observation. TA-BSA@CuS (50 μg/mL) were mixed with the same amounts of bacteria and divided into NIR (+) and NIR (-) groups. Subsequently, the NIR (+) group was irradiated with NIR (1064 nm, 1.0 W/cm^2) for 10 min, then incubated in 37 °C for 6 h, the NIR (-) group was also prepared without irradiation. After being incubated, we used the OD600 value of the bacterial suspension to determine the bacterial count and the dilution multiple. The bacterial suspensions were diluted to 10^3 CFU/mL and cultured on agar plates overnight to calculate the number of live bacteria cells.

For the SEM observation, the bacteria were immobilized by 2.5% glutaraldehyde overnight. After that, the samples were dehydrated using graded ethanol-water solutions. Then the dehydrated bacteria cells were observed by SEM.

Intracellular ROS Scavenging

An ROS assay kit (Beyotime) was used to determine intracellular ROS levels. Specifically, the cells were seeded in 6-well plates (6×10^3 cells/well) and treated with the oxalate (0.7 mM). After 24 h of incubation at 37 °C in a humidified atmosphere containing 5% CO_2, culture medium in each well was replaced by fresh DMEM or containing TA-BSA@CuS (50 μg/mL) and incubated for a certain time. The prepared 6-well plate were divided into NIR (+) and NIR (-) groups. The NIR (+) group was irradiated by NIR (1064 nm, 1.0 W/cm^2) for 10 min, and the NIR (-) group was incubated without NIR. After treatment, the cells were loaded with 1 mL of a DMEM/F12 and 2',7'-dichlorofluorescein diacetate mixed solution (DCFH-DA, 10 μM) for 20 min at 37 °C. Then the cells

were collected, washed, and resuspended in PBS (100 μL). Flow cytometry was performed with excitation and emission at 488 nm and 525 nm respectively to determine intracellular ROS.

RESULTS AND DISCUSSION

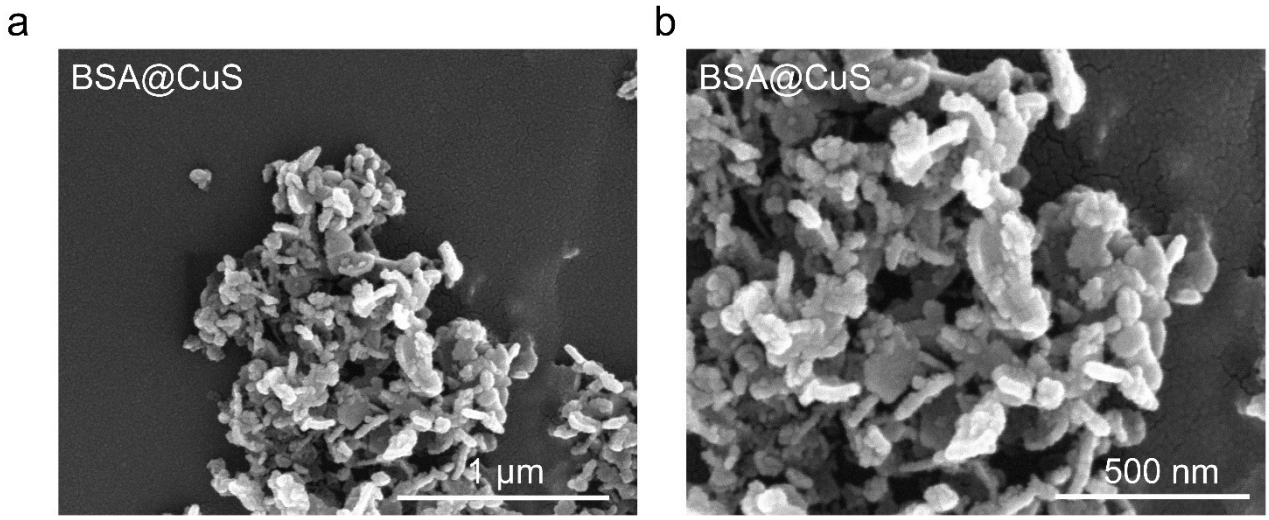

Figure S1. SEM images of BSA@CuS.

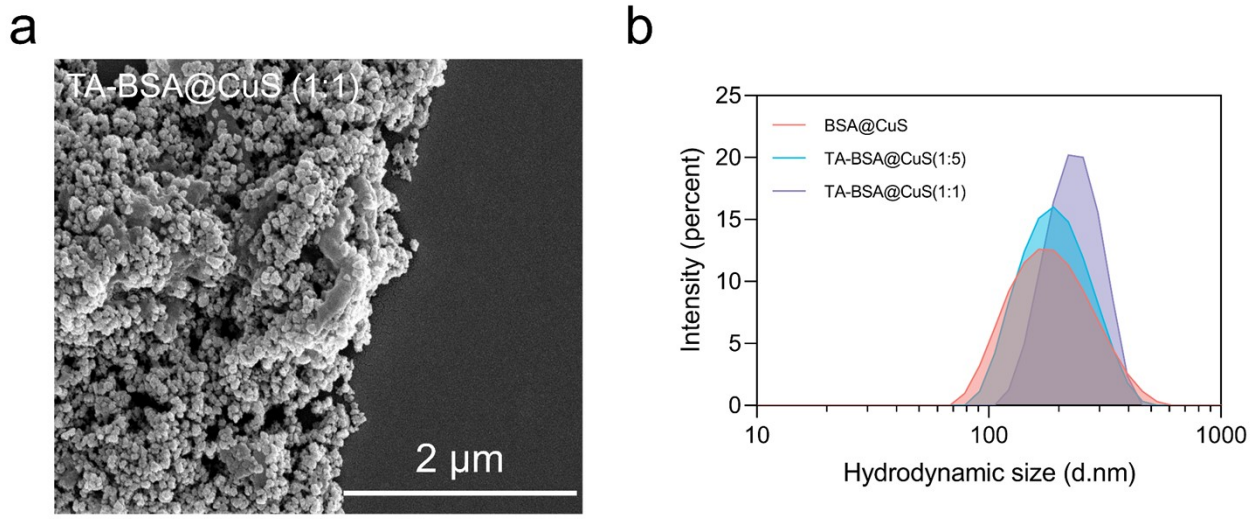

Figure S2. (a) SEM image of the TA-BSA@CuS at the ratio of TA to Cu of 1:1. (b) Hydrodynamic size distribution for different TA to Cu^{2+} ratios.

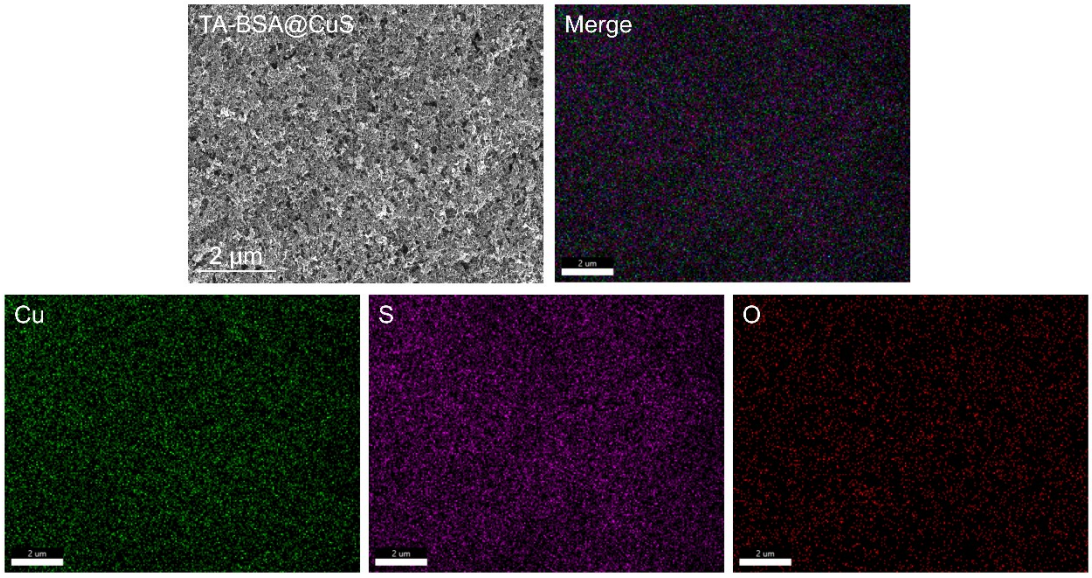

Figure S3. SEM images and corresponding EDS elemental mapping for the distribution of Cu, S, and O elements of TA-BSA@CuS.

Table S1. Element atomic qualification results of Cu, S, and O elements of BSA@CuS and TA-BSA@CuS by SEM mapping analysis.

Sample	**Element**		
	Cu (Atom %)	S (Atom %)	O (Atom %)
BSA@CuS	18.17	12.99	8.00
TA-BSA@CuS	18.40	14.18	5.63

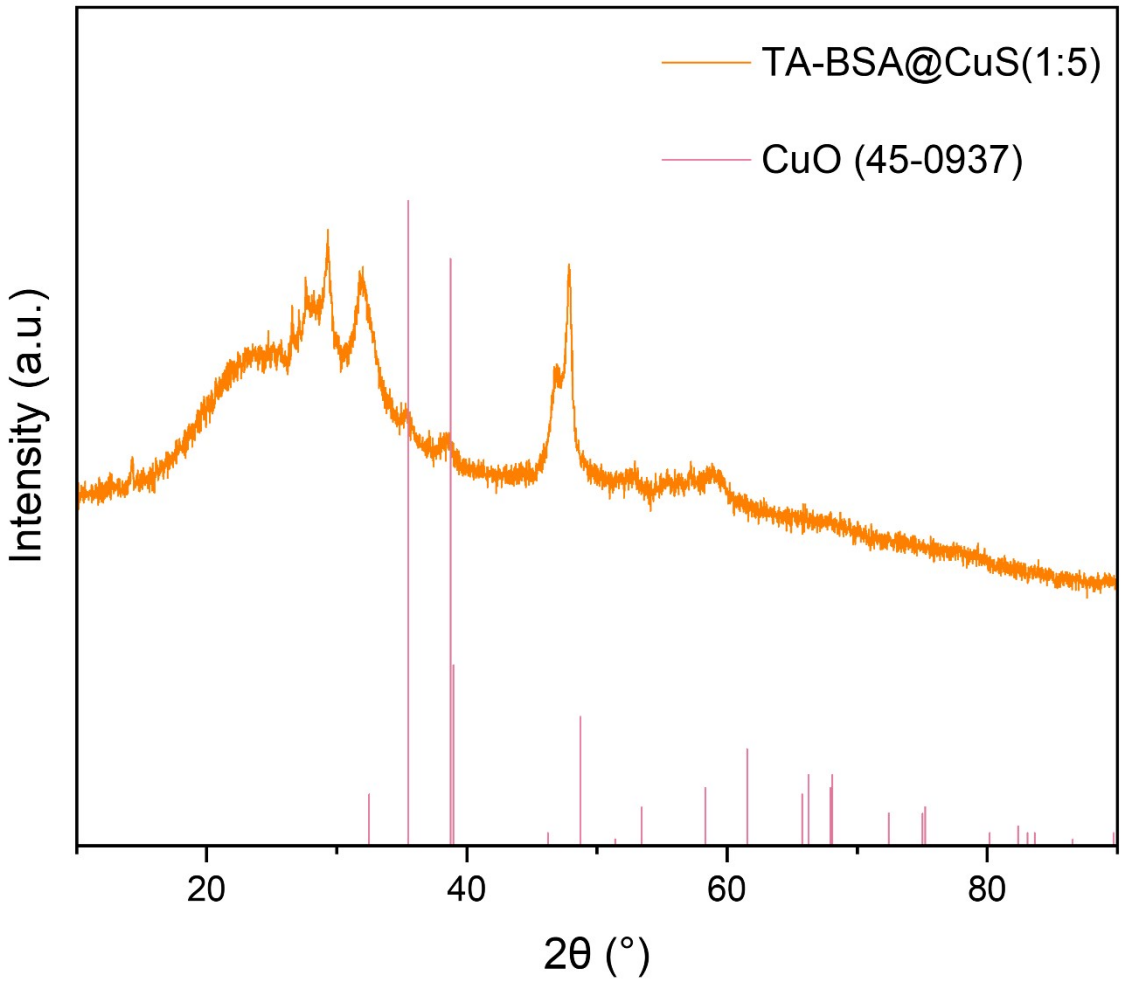

Figure S4. Power X-ray diffraction (XRD) patterns of TA-BSA@CuS (1:5) and CuO (JCPDS#45-0937) (The ratio is TA to Cu^{2+}).

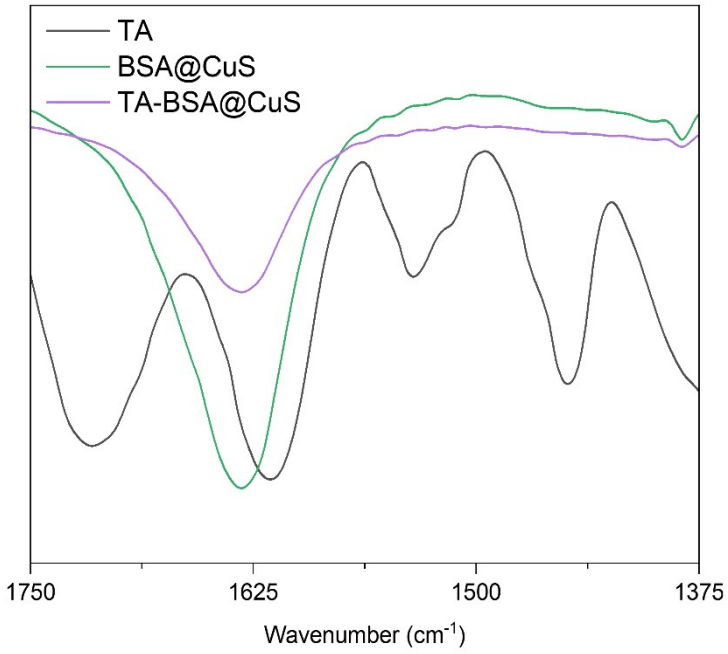

Figure S5. The FTIR spectra of TA, BSA@CuS and TA-BSA@CuS local enlargements between 1375 cm^{-1} and 1750 cm^{-1}.

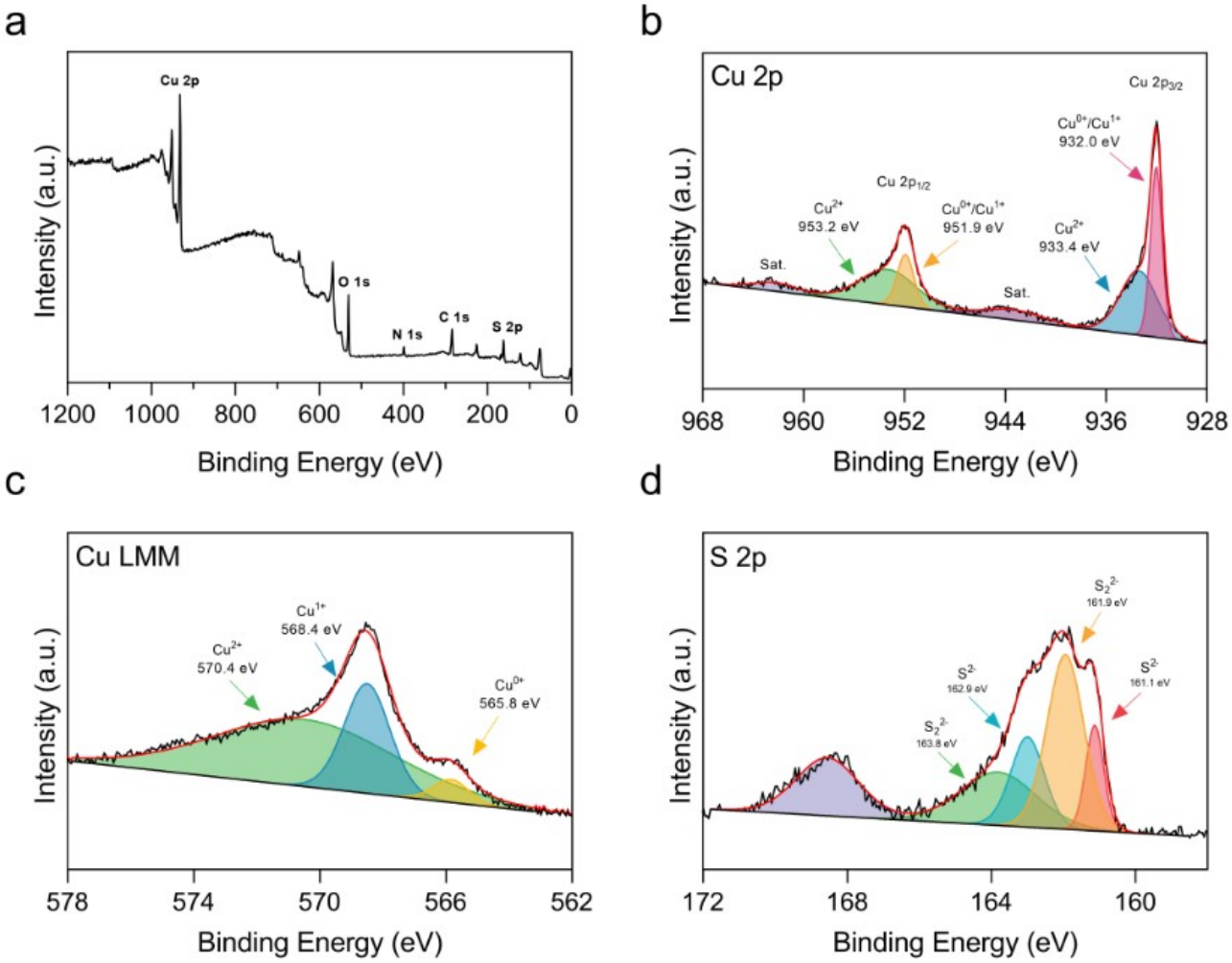

Figure S6. (a) XPS survey spectra, (b) high-resolution Cu 2p XPS spectra, (c) high-resolution Cu LMM Auger XPS spectra, and (d) high-resolution S 2p XPS spectra of BSA@CuS.

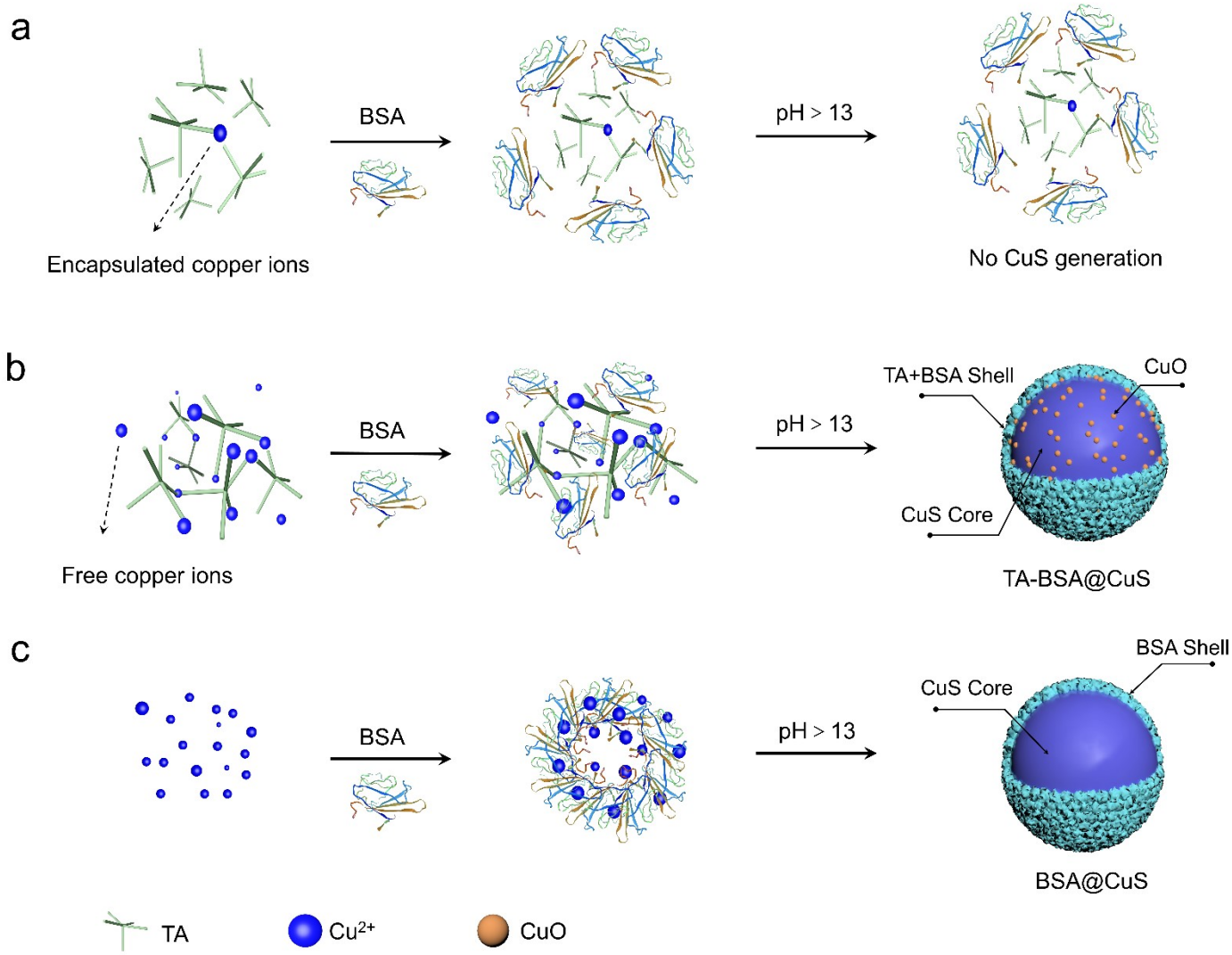

Figure S7. Schematic representation for the effect of polyphenols (TA) towards the synthetic processes of TA-BSA@CuS, (a) numerous TA, (b) a proper content of TA, and (c) little or no TA.

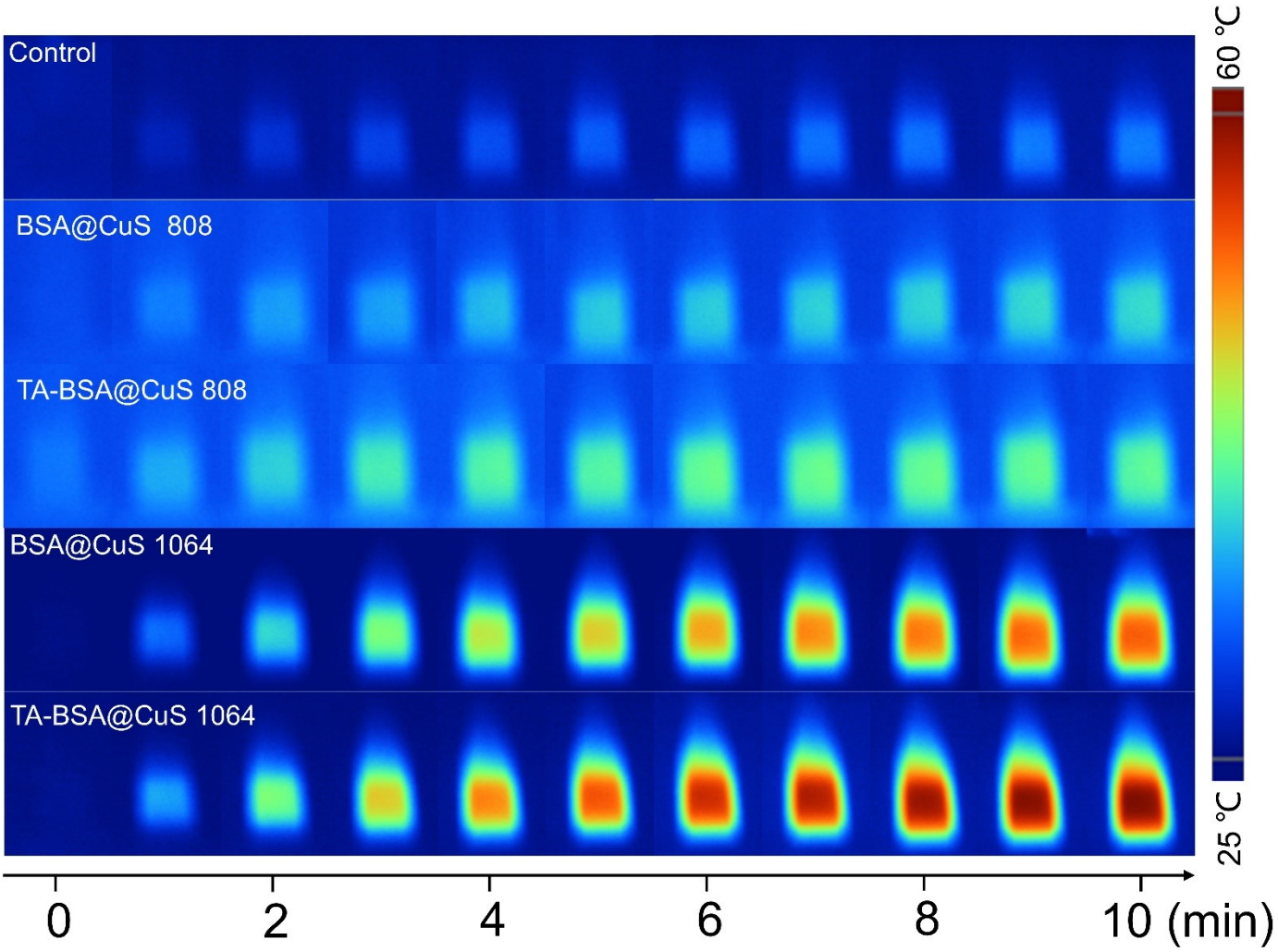

Figure S8. Photothermal images for the BSA@CuS (50 μg/mL) and TA-BSA@CuS (50 μg/mL) with 808 nm and 1064 nm laser (1.0 w/cm^2, 10 min).

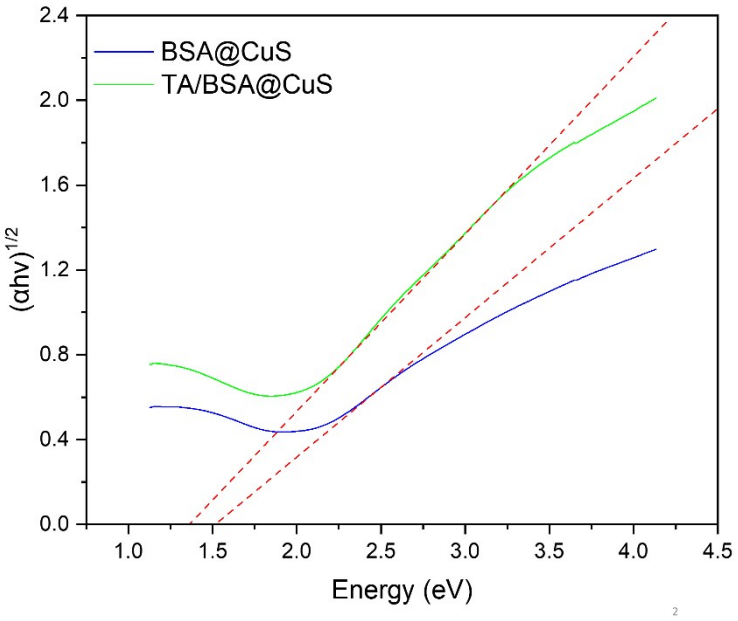

Figure S9. Tauc plot absorption spectrum method for calculation of bandgap.

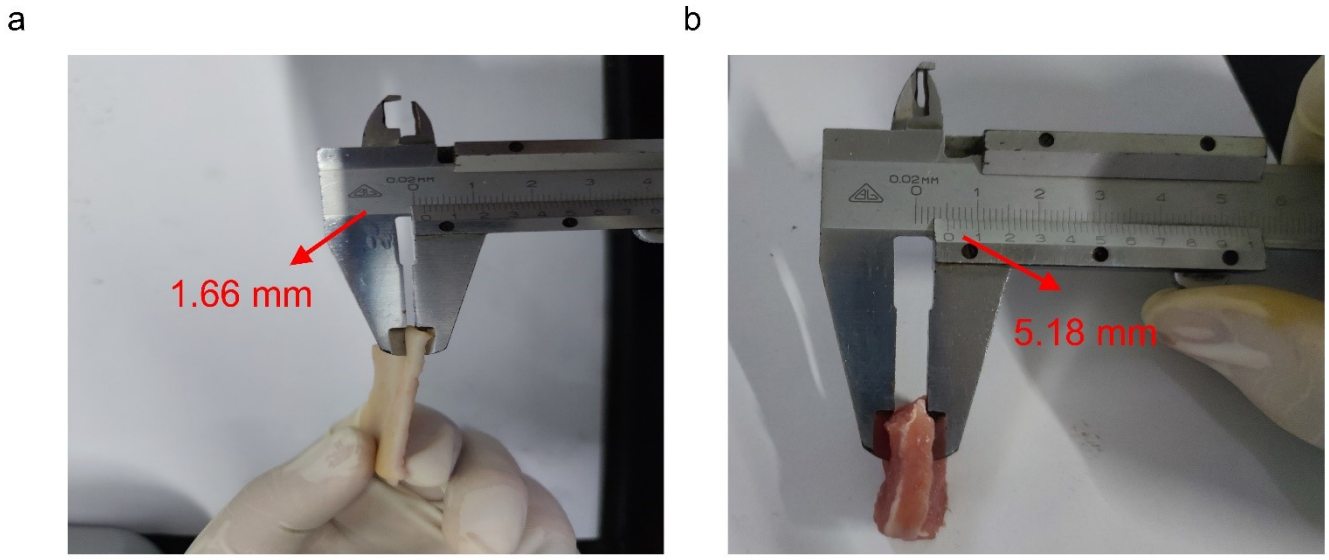

Figure S10. Size images of (a) pig skin and (b) pork.

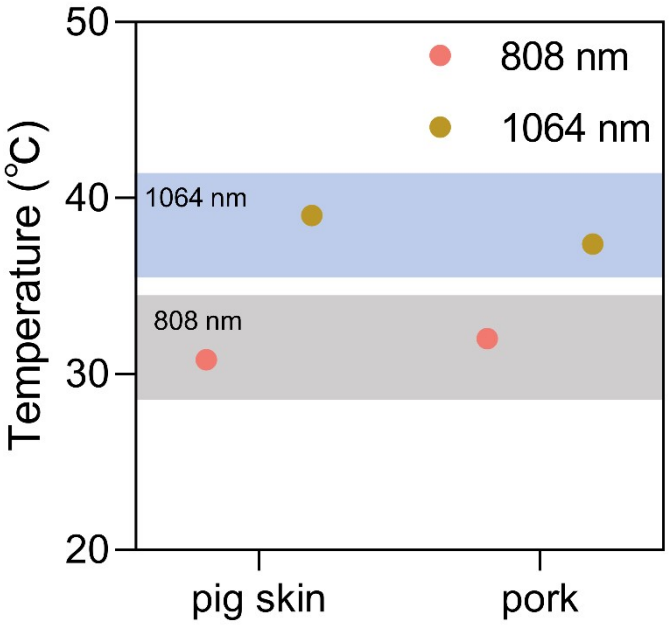

Figure S11. Temperature changes of TA-BSA@CuS treated with pig skin and pork with different NIR-II light.

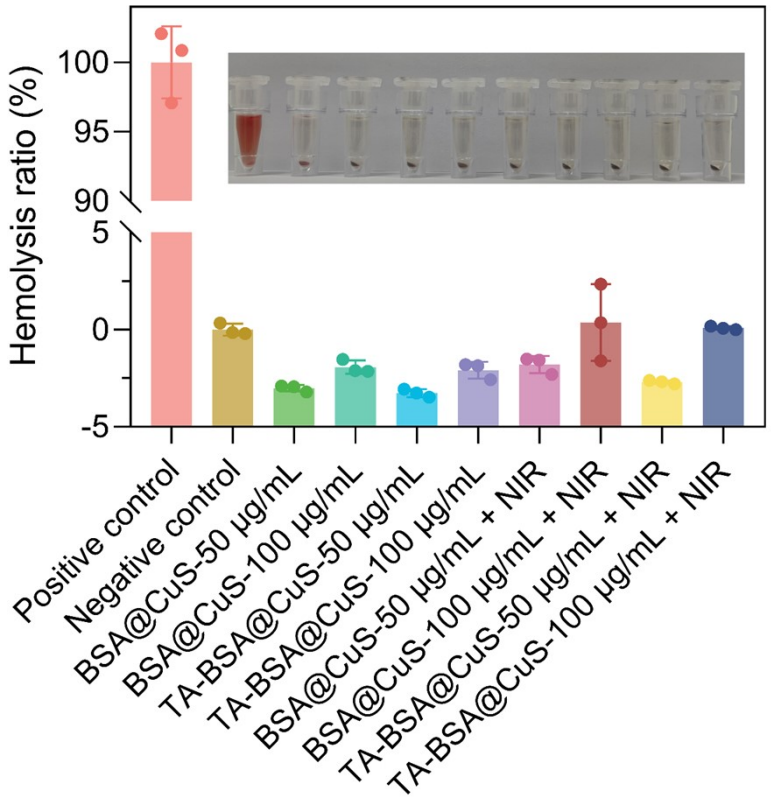

Figure S12. Digital photos of hemolysis for different samples, the photos are in the same order as the abscissa.

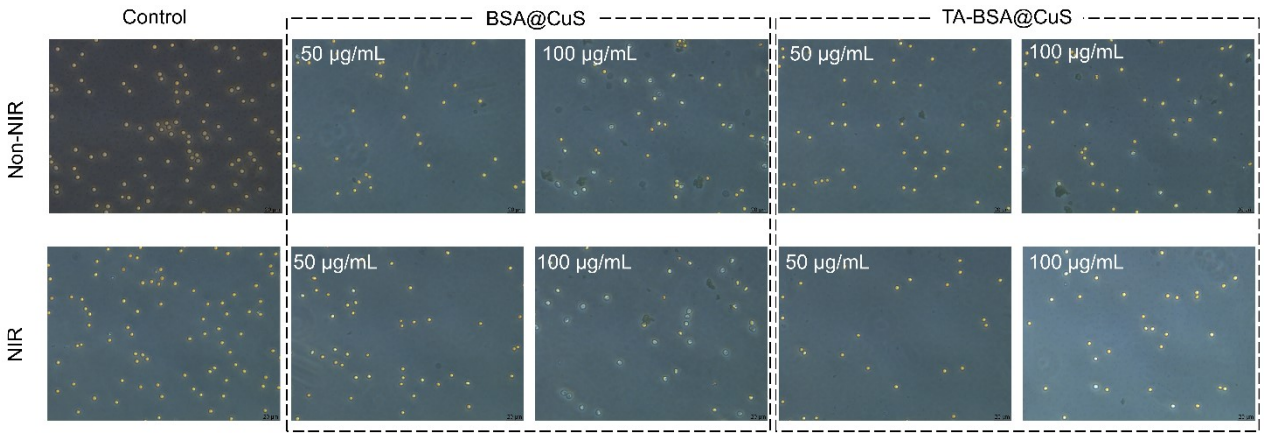

Figure S13. Representative microscope images for RBCs treated with PBS, BSA@CuS, or TA-BSA@CuS.

Table S2. Descriptive statistics outcome of blood routine test.

	Samples	Mean	Std. Deviation	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Erythrocyte (10^{12}/L)	Blank	4.323	0.058	4.258	4.389
	BSA@CuS	4.313	0.032	4.277	4.350
	TA-BSA@CuS	4.337	0.038	4.294	4.380
Leukocyte (10^{9}/L)	Blank	4.953	0.116	4.822	5.084
	BSA@CuS	5.080	0.044	5.031	5.129
	TA-BSA@CuS	5.077	0.090	4.975	5.178
Platelet (10^{9}/L)	Blank	187.000	6.557	179.580	194.420
	BSA@CuS	183.667	11.060	171.151	196.183
	TA-BSA@CuS	188.667	6.658	181.132	196.201

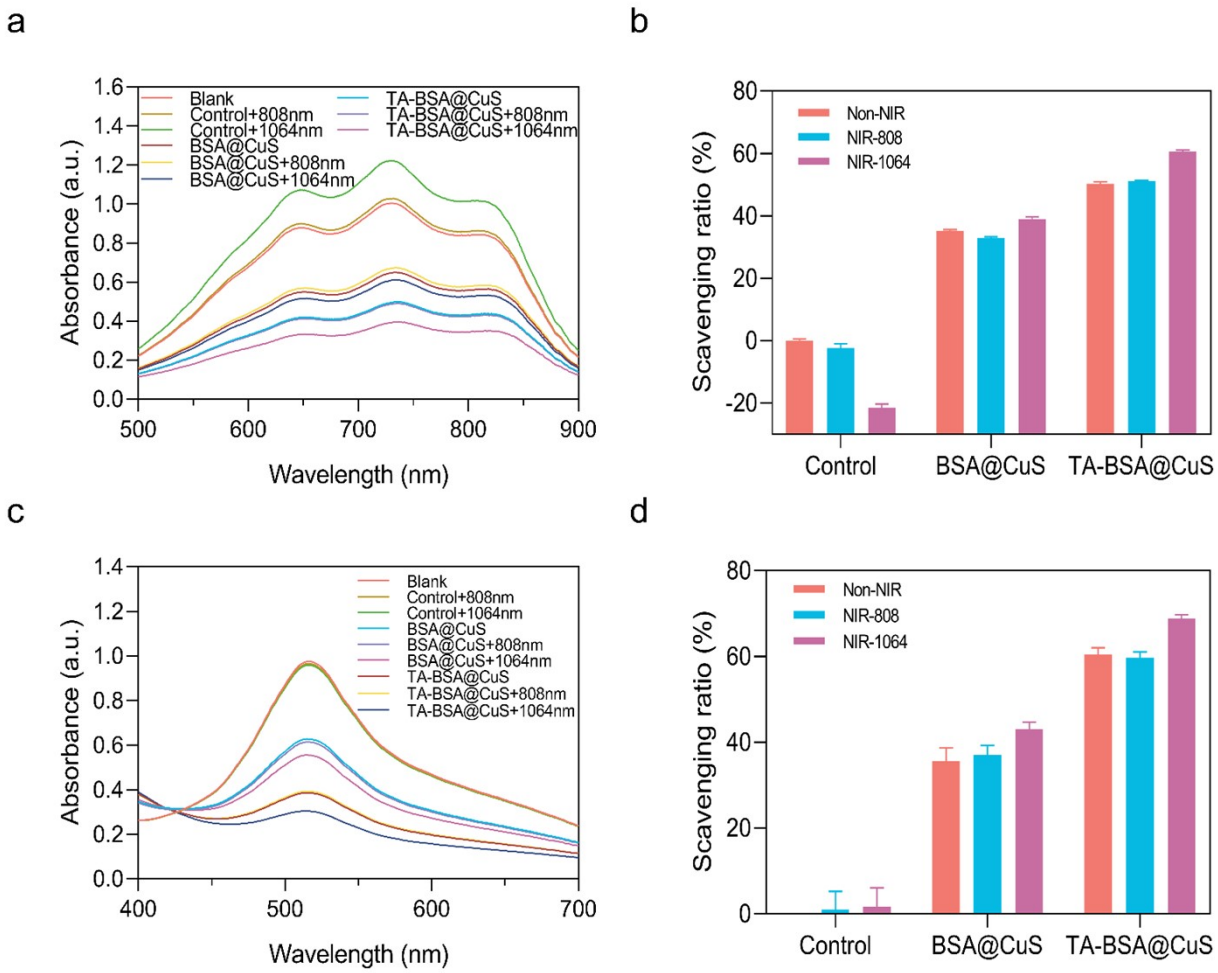

Figure S14. RNS scavenging activity of BSA@CuS or TA-BSA@CuS. (a) $ABTS^{\bullet+}$ solutions, (b) $ABTS^{\bullet+}$ scavenging rate, (c) $DPPH^{\bullet}$ solutions, and (d) $DPPH^{\bullet}$ scavenging rate with/without NIR.

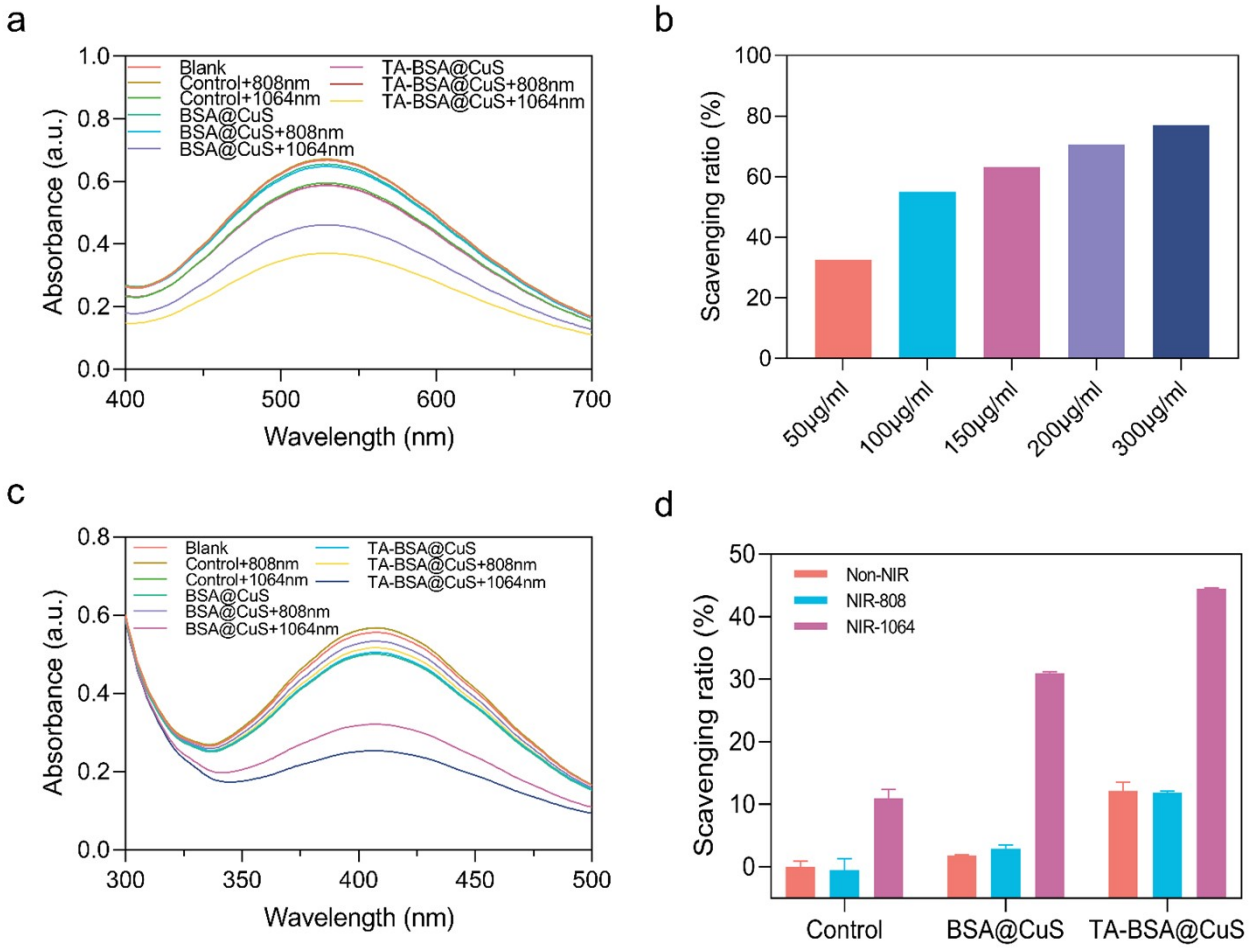

Figure S15. ROS scavenging activity of BSA@CuS or TA-BSA@CuS. (a) •OH concentration, (b) •OH scavenging rate, (c) H_2O_2 solutions and (d) H_2O_2 scavenging rate with/without NIR.

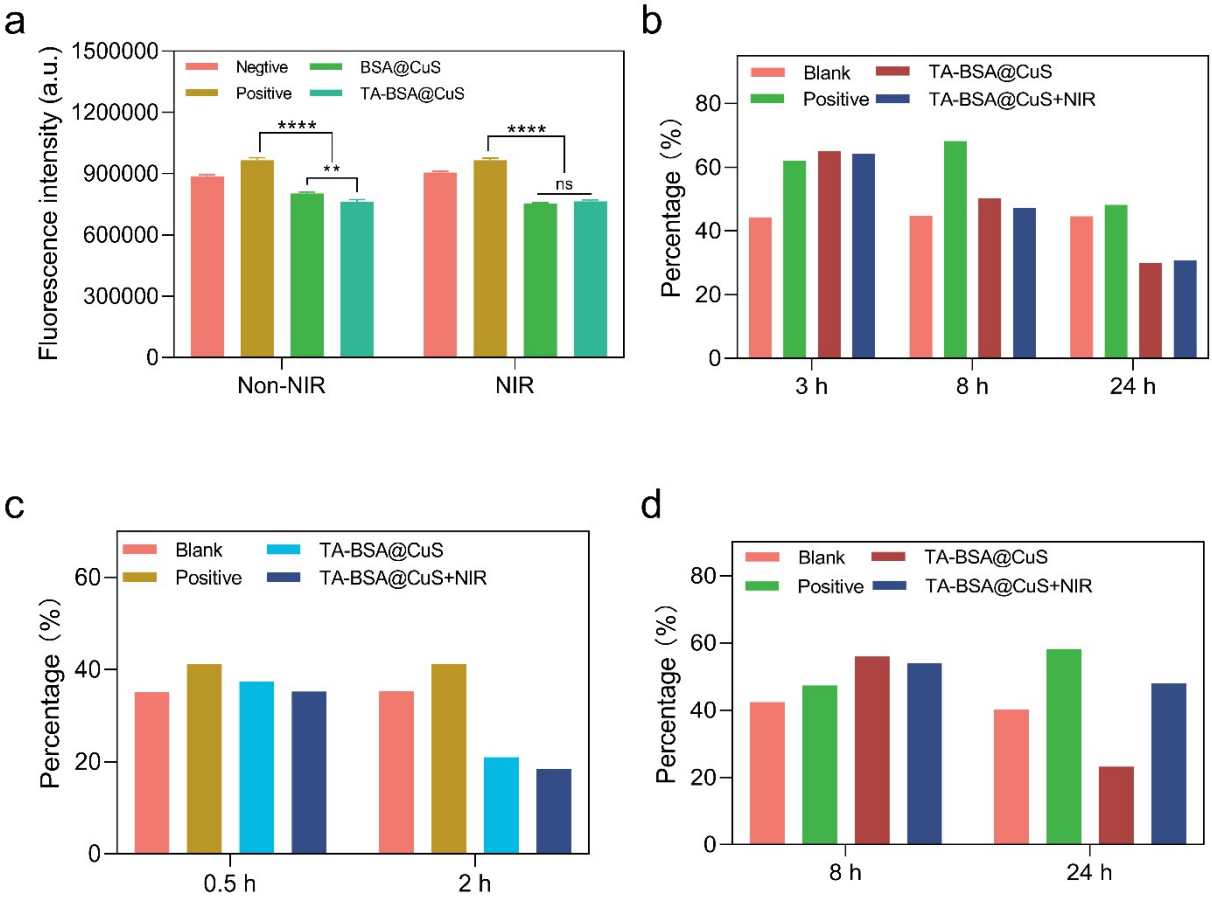

Figure S16. Antioxidant effect of TA-BSA@CuS in HK-2. (a) Fluorescence intensity at 24h incubation after irradiation. ROS levels in the cellular environment were monitored by flow cytometry analysis (b) with different times of incubation after irradiation, and (c, d) irradiated after different times of incubation.

Reference

1. Wei, Z.; Wang, L.; Tang, C.; Chen, S.; Wang, Z.; Wang, Y.; Bao, J.; Xie, Y.; Zhao, W.; Su, B.; Zhao, C., Metal-Phenolic Networks Nanoplatform to Mimic Antioxidant Defense System for Broad-Spectrum Radical Eliminating and Endotoxemia Treatment. *Adv. Funct. Mater.* **2020,** *30* (49), 2002234.