

Supporting Information

Catalytic and photoresponsive BiZ/Cu_xS heterojunctions with surface vacancies for the treatment of multidrug-resistant clinical biofilm-associated infections

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Materials

Bismuth nitrate pentahydrate ($\text{Bi}(\text{NO}_3)_3 \cdot 5\text{H}_2\text{O}$), nitric acid (HNO_3 , 65%), crystal violet dye, and bovine serum albumin (BSA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Copper sulfate anhydrous (CuSO_4) was purchased from Kanto Chemical (Tokyo, Japan). Sodium phosphate monobasic monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$), sodium phosphate dibasic heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$), and sodium sulfide non-hydrate ($\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$) were acquired from Acros Organics (Geel, Belgium). 10–Acetyl–3,7–dihydroxyphenoxazine (Amplex Red) was obtained from Invitrogen (Carlsbad, CA, USA). Hydrogen peroxide (aqueous solution, 35%) was purchased from Showa Chemical Industries Co. Ltd., Tokyo, Japan, respectively. Sodium hydroxide (NaOH) was bought from J.T. Baker (Center Valley, PA, USA). Bacterial strains [*S. aureus* (BCRC10781), *S. enteritidis* (BRBC 10744), *E. coli* (BRBC 12438), *P. aeruginosa* (ATCC 27853), and MRSA (methicillin–resistant *Staphylococcus aureus* (ATCC 43300)] were procured from the Institute of Food Science (Hsinchu, Taiwan). Milli–Q ultrapure water (18.2 M Ω cm) from Millipore (Billerica, MA, USA) was used in all experiments.

Characterization of BSA–BiZ/Cu_xS, –Cu_xS, and –Bi₂S₃ nanomaterials

Solutions were purified using centrifugation (3 washing cycles) to remove unreacted metal ions (Cu^{2+} and Bi^{3+}), free BSA, and excess NaOH. Final concentrations of Cu and Bi in BSA–BiZ/Cu_xS NCs, BSA–Cu_xS NPs, and BSA–Bi₂S₃ NPs separately dissolved in 2.0 % HNO_3 solution were determined by inductively coupled plasma mass spectrometry (ICP–MS; Agilent 7700 Series, Agilent Technologies, Santa Clara, CA, USA). The X-ray source (SP12B1; energy range: 8–25 KeV) from a bending magnet was used to record the diffraction patterns for BSA–BiZ/Cu_xS NCs, BSA–Cu_xS NPs, and BSA–Bi₂S₃ NPs, which were recorded using a CCD detector (BL12B2, Spring–8, and a 3–pin plate diamond anvil cell) from Almax easyLab (Almax Industries, Diksmuide, Belgium). The two–dimensional images were then integrated using the FIT2D proGram, with “Appendix” to set the wavelength corresponding to Cu K α (1.5418 Å). High–resolution transmission electron microscopy (HR–TEM) images of BSA–BiZ/Cu_xS NCs, BSA–Cu_xS NPs, and BSA–Bi₂S₃ NPs were captured using field emission transmission electron microscopy (FE–TEM) equipped with dual focused ion beam system (FIB) and energy

dispersive X-ray spectrometer (EDS). UV-Vis absorption spectra of the BSA-BiZ/Cu_xS NCs, BSA-Cu_xS NPs, and BSA-Bi₂S₃ NPs in the range of 200–1400 nm was measured using a double-beam Jasco UV/VIS/NIR spectrophotometer (V-570, Easton, MD, USA). Each of the solution was diluted 10,000-fold in DI water, from which 2.0 μL was dropped onto a carbon-coated grid and vacuum dried for 48 h at room temperature before measurements. The binding energies of the elements C, O, Cu, Bi, and S were obtained from X-ray photoelectron spectroscopy (XPS, VG ESCA210) from VG Scientific (West Sussex, UK). All the binding energies were corrected using C 1s (284.6 eV) as an internal standard. Aliquots (50 μL) of 10-fold concentrated BSA-BiZ/Cu_xS NCs and BSA-Cu_xS NPs solutions were simply dropped onto Si substrate and vacuum dried at room temperature for XPS analysis. Laser desorption/ionization time-of-flight mass spectrometry (LDI-TOF MS) was used to identify the possible components in BSA-BiZ/Cu_xS NCs and BSA-Cu_xS NPs using AutoflexIII S3 LDI-TOF MS (Bruker Daltonics, Bremen, Germany). Each of the sample was irradiated with a Nd:YAG pulse laser (355 nm, 100 Hz; 500 shots, pulse width: 6 ns, power density: 5 × 10⁴ W cm⁻²). Each of the MS signals was accumulated from five target positions. Fourier transform infrared (FTIR) spectroscopy analysis for BSA-BiZ/Cu_xS NCs and BSA-Cu_xS NPs were performed with Nicolet iS5 FTIR spectrometer (Thermo Scientific, Waltham, MA, USA). Their circular dichroism (CD) spectra in a cuvette of path length 1cm were recorded using J-815 spectrometers (JASCO, Tokyo, Japan) at room temperature.\

Bacterial viability assays

Bacterial suspensions of *E. coli* (1.0 × 10⁸ CFU mL⁻¹) were incubated separately with BSA-BiZ/Cu_xS NCs ([Cu] = 0.2 μg mL⁻¹) and BSA-Cu_xS NPs ([Cu] = 0.2 μg mL⁻¹) dispersed in 10 mM sodium phosphate buffer (pH 7.4) for 10 min with and without NIR laser irradiation (808 nm, 0.53 W cm⁻²) at room temperature. The untreated and treated bacterial samples were centrifuged (RCF 3000 × g, 5 min, 27 °C) and washed thrice with 10 mM sodium phosphate buffer (pH 7.4, 1.0 mL) prior to fluorescence assessment. A LIVE/DEAD BacLight Bacterial Viability Kit (Molecular Probes, Eugene, OR, USA) was used to quantify live/dead bacteria ratio in each of the tested sample. Typically, 1.0 μL of SYTO 9:PI (1:1) was added into each of the treated and untreated (negative control) bacterial samples, followed by incubation in the dark for 20 min at room temperature. Dye-suspensions were then centrifuged (RCF 3000 × g, 5 min, 27 °C) to remove the unbound dyes, and washed thrice with sodium phosphate buffer

(10 mM, pH 7.4). Aliquots (1.0 μL) of the purified solutions were dropped onto the glass slide for bright field and fluorescence microscopic analysis. The green and red fluorescence observed through emission filters of ≥ 525 nm, when excited with an Hg lamp with an excitation filter 460–490 nm (Olympus 1X71 microscope, Tokyo, Japan) represents the live and dead bacteria, respectively.

Reactive oxygen species (ROS) assays

Bacterial culture of *E. coli* (1.0×10^8 CFU mL^{-1}) were treated separately with 0.25 $\mu\text{g mL}^{-1}$ (in terms of Cu) of BSA–BiZ/ Cu_xS NCs and BSA– Cu_xS NPs dispersed in sodium phosphate buffer (10 mM, pH 7.4) in absence and presence of NIR laser (808 nm, 0.53 W cm^{-2}) at room temperature for 10 min. The untreated and hydrogen peroxide (H_2O_2 ; 10 mM)–treated bacteria culture served as the negative and positive controls, respectively. Each of the mixture was then centrifuged (RCF 3000 $\times g$, 5 min, 27 $^\circ\text{C}$) and washed thrice with 10 mM sodium phosphate buffer (pH 7.4). Subsequently, 2',7'–Dichlorodihydrofluorescein diacetate (DCFH–DA, 100 μM) was separately added to each of the bacterial sample and incubated for 2 h at room temperature. Then, an aliquot (200 μL) of the solution from each vial was transferred into a 96–well flat–bottom microplate to measure the fluorescence intensities at an excitation/emission wavelengths of 490/530 nm, respectively, using a monochromatic microplate spectrophotometer.

Permeability of bacterium membrane

Bacterial suspensions of *E. coli* (1.0×10^8 CFU mL^{-1}) were incubated separately with BSA–BiZ/ Cu_xS NCs ($[\text{Cu}] = 4.0 \mu\text{g mL}^{-1}$) and BSA– Cu_xS NPs ($[\text{Cu}] = 4.0 \mu\text{g mL}^{-1}$), with and without NIR laser irradiation (808 nm, 0.53 W cm^{-2}) for 10 min at room temperature. PBS and B–PER lysis buffer treated aliquots were used as positive and negative control, respectively. Each of the solutions (1.0 mL) was centrifuged at an RCF of 3000 $\times g$ for 5 min. The supernatants were then incubated separately with 200 μM *o*-nitrophenyl- β -D-galactopyranoside (ONPG) at room temperature for 2 h with orbital shaking. Lastly, 200 μL of each solution was transferred in a 96-well flat-bottom microplate to record its absorbance at 420 nm by using a monochromatic microplate spectrophotometer (Synergy 4, Biotek Instruments, Winooski, VT, USA).

SEM and TEM images of bacteria

1.0 mL of bacteria suspension (*E. coli*; 1.0×10^8 CFU mL⁻¹) was centrifuged (RCF 3000 × g, 5 min, 27 °C) and then dispersed in 10 mM sodium phosphate buffer (pH 7.4, 1 mL). Bacterial culture (1×10^7 CFU mL⁻¹) of *E. coli* was untreated (negative control) and separately incubated with 4.0 μg mL⁻¹ (in terms of Cu) of BSA–BiZ/Cu_xS NCs and BSA–Cu_xS NPs with and without NIR laser irradiation (808 nm, 0.53 W cm⁻²) for 10 min at room temperature. The mixtures were then purified by centrifugation (RCF 3000 × g, 5 min, 27 °C) and washed with 10 mM sodium phosphate buffer (pH 7.4, 1.0 mL) before the pellets were suspended in 10 mM sodium phosphate buffer (pH 7.4, 1.0 mL). Then, the cells were fixed using 1.0 % glutaraldehyde at room temperature for 30 min. Afterwards, the bacteria solutions were centrifuged (RCF 3000 × g, 5 min, 27 °C) and redispersed in 10 mM sodium phosphate buffer (pH 7.4). Finally, 5 μL (1×10^5 CFU mL⁻¹) from each of the resuspended solution was immobilized and vacuum dried (2 h, 27 °C) on a Si substrate and copper–coated carbon grid for SEM and TEM analysis, respectively.

In vitro cytotoxicity assays

The cytotoxicity of BSA–BiZ/Cu_xS NCs and BSA–Cu_xS NPs were investigated using Alamar blue assay (Thermo Fisher Scientific Inc.). Briefly, HaCaT cells (aneuploid immortal keratinocyte cell line from adult human skin, ATCC, Manassas, VA, USA) were seeded in 96–well plates (1×10^4 cells/well) and cultured for 12 h at 37 °C in a humidified incubator. Various concentrations ([Cu] = 0–80 μg mL⁻¹) of BSA–BiZ/Cu_xS and BSA–Cu_xS NPs NCs were incubated separately with the cells for 24 h at 37 °C. The nanomaterials were then replaced with a fresh medium for further growth for 24 h at 37 °C. Afterwards, the culture media were removed and cells were incubated with the Alamar blue solution (100 μL, 1x in DMEM plus 10% FBS) at 37 °C for 2 h. The fluorescence intensity of each well was measured using a microplate reader at an excitation/emission wavelengths of 550/600 nm, respectively. The cell viability (%) was determined as per the following equation:

Cell viability (%) = $(F_{\text{Test}} / F_{\text{Blank}}) \times 100\%$, where, F_{Test} and F_{Blank} are the fluorescence intensities of the cells in the presence and absence of nanomaterials.

Hemolysis assays

A blood sample was withdrawn from the vein of a 28-year-old healthy male and stored in a sterile test tube containing trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$). The red blood cell (RBCs) collection protocols employed were according to the university guidelines and relevant laws. The blood sample was promptly centrifuged ($3000 \times g$, 10 min, 4°C) to remove the serum, and washed three times with PBS solution (pH 7.4). Various amounts ($[\text{Cu}] = 0\text{--}80 \mu\text{g mL}^{-1}$) of BSA–Bi/ Cu_xS NCs and BSA– Cu_xS NPs were incubated separately with RBCs (~ 4 vol.% of blood cells) containing PBS (pH 7.4) for 1 h at room temperature. The mixtures were centrifuged (RCF $3000 \times g$, 10 min, 4°C) and the supernatants were subjected to absorption measurement at 576 nm (Abs_{576}). RBCs treated separately with PBS (1x, pH 7.4) and DI water served as negative (0% hemolysis, $\text{Abs}_{576 \text{ blank}}$) and positive (100% hemolysis, $\text{Abs}_{576 \text{ water}}$) controls, respectively. The hemolysis (%) was determined using the following formula:

$$\text{Hemolysis (\%)} = [(\text{Abs}_{576 \text{ Test}} - \text{Abs}_{576 \text{ Blank}}) / (\text{Abs}_{576 \text{ Water}} - \text{Abs}_{576 \text{ Blank}})]$$

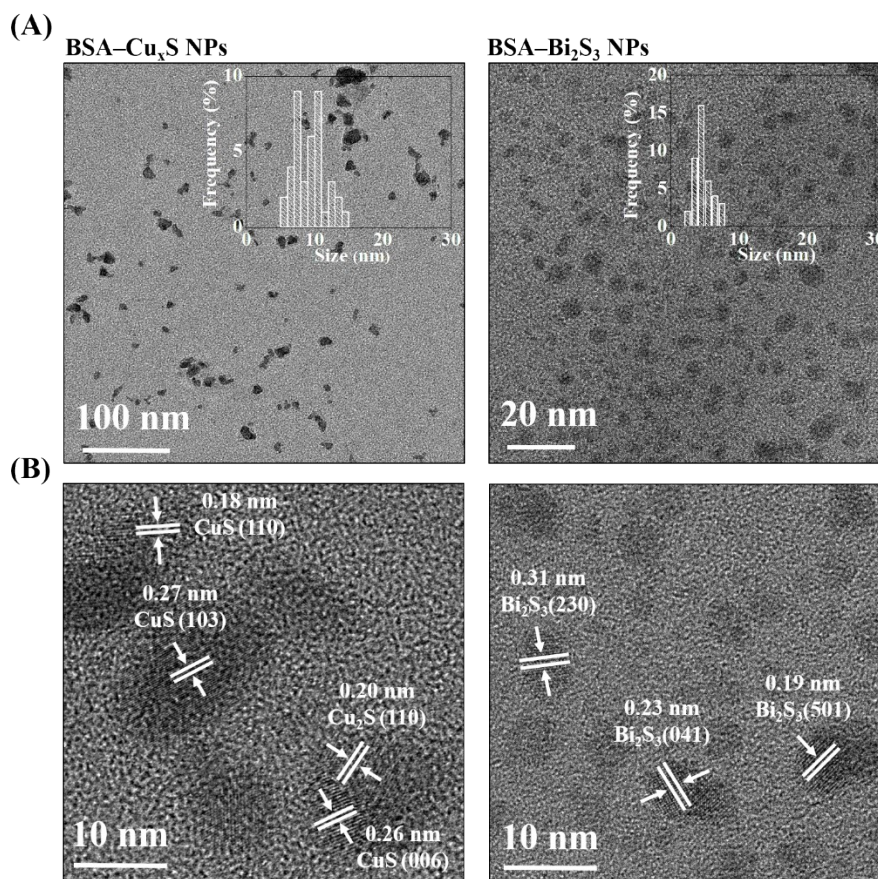


Fig. S1 (A) TEM and (B) corresponding HR-TEM images of BSA-Cu_xS NPs and BSA-Bi₂S₃ NPs. The insets to (A) show the particle size distribution.

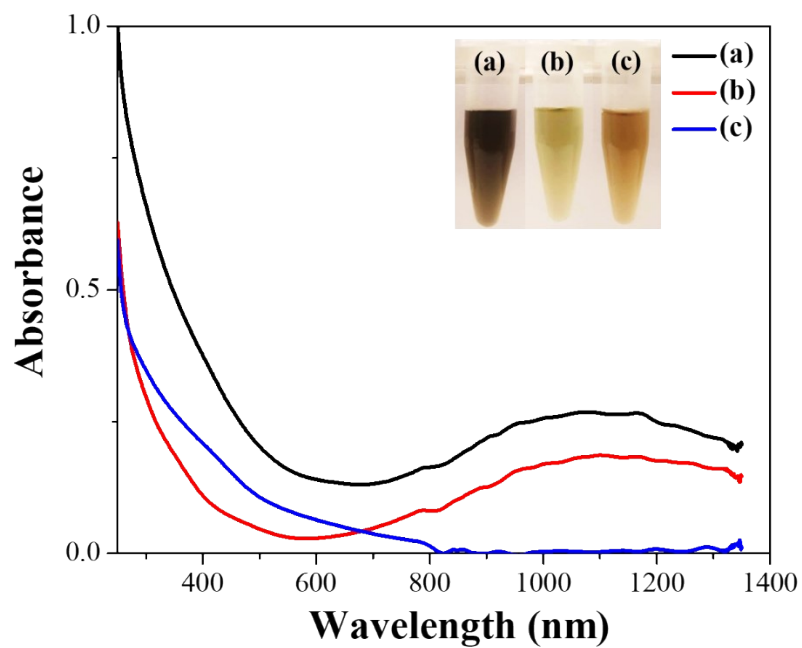


Fig. S2. UV-Vis-NIR absorption spectra of (a) BSA–BiZ/Cu_xS NCs (b) BSA–Cu_xS NPs, and (c) BSA–Bi₂S₃ NPs, with 20-fold dilution of as-prepared solutions in 10 mM sodium phosphate buffer (pH 7.4). Insets show the digital photographs of the corresponding solutions.

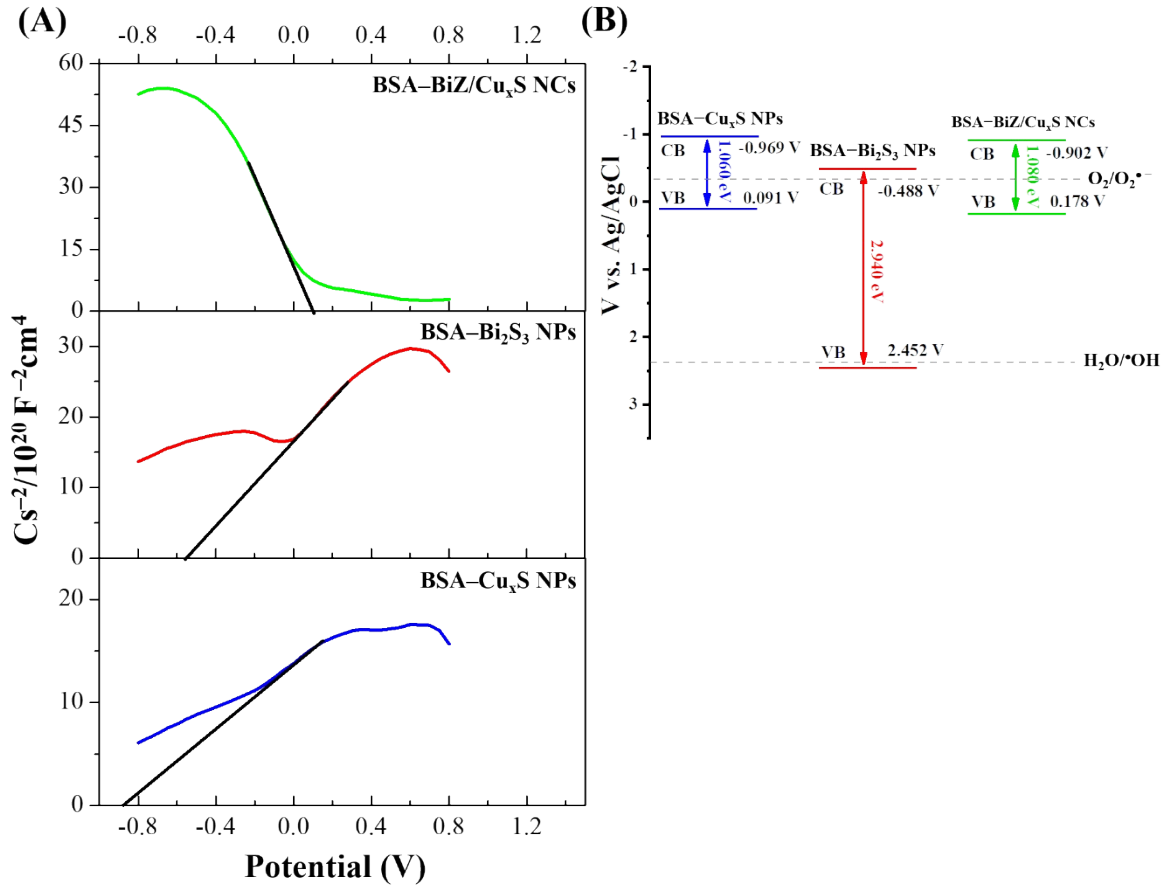


Fig. S3. (A) Mott-Schottky plots for the BSA-BiZ/Cu_xS NCs, BSA-Cu_xS NPs, and BSA-Bi₂S₃ NPs in 0.1 M sodium sulfate (Na₂SO₄). (B) Energy band diagram based on the determined flat band potential.

We use Mott-Schottky (M-S) plots to determine the position of the band edges of the BSA-BiZ/Cu_xS NCs, BSA-Cu_xS NPs, and BSA-Bi₂S₃ NPs. The carrier density (N_d) and flat band potential (V_{FB}) of the materials is calculated using the well-known M-S equation (1).¹

$$\frac{1}{C^2} = \left(\frac{2}{N_d \epsilon \epsilon_0 e_0 A^2} \right) \times \left(V - V_{FB} - \frac{k_B T}{e_0} \right) \quad (1)$$

where N_d is carrier donor density (cm³), C is space charge capacitance, ϵ is the dielectric constant of nanomaterials, ϵ_0 the vacuum permittivity ($8.85 \times 10^{-14} \text{ F m}^{-1}$), A is working area of electrode (GCE; 0.0729 cm^2), V is applied potential, V_{FB} is flat-band potential, k_B is Boltzmann constant ($1.38 \times 10^{-23} \text{ m}^2 \text{ kg s}^{-2} \text{ K}^{-1}$), T is absolute temperature (K), and e_0 is the electric charge ($1.60 \times 10^{-19} \text{ C}$). The V_{FB} can be estimated from the x-intercept of the M-S plot (V versus $1/C^2$). In contrast, the N_d of the semiconductor at the semiconductor-liquid interface is calculated from the corresponding slope (Δ) expressed in the following:

$$N_d = \left(\frac{2}{\epsilon \epsilon_0 e_0} \right) \times \Delta \quad (2)$$

The value of V_{FB} given in equation (1) approximately refers to the valence band edge (V_{VB}) potentials and conduction band edge (V_{CB}) in the case of n and p -type semiconductors, respectively. Tauc plot shows the bandgap energies of the as-prepared BSA-Cu_xS NPs, BSA-Bi₂S₃ NPs, and BSA-BiZ/Cu_xS NCs are 1.060, 2.940, and 1.080 eV, respectively. The flat-band potential determined for BSA-BiZ/Cu_xS NCs (0.178 V) is positively shifted relative to BSA-Cu_xS NPs (-0.969 V) and BSA-Bi₂S₃ NPs (-0.488 V) in Na₂SO₄ solution. According to flat-band potentials calculated by M-S plots, the CB/VB positions of BSA-Cu_xS NPs, BSA-Bi₂S₃ NPs, and BSA-BiZ/Cu_xS NCs, were estimated to be -0.969/0.091, -0.488/2.452, and -0.902/0.178 eV, respectively.

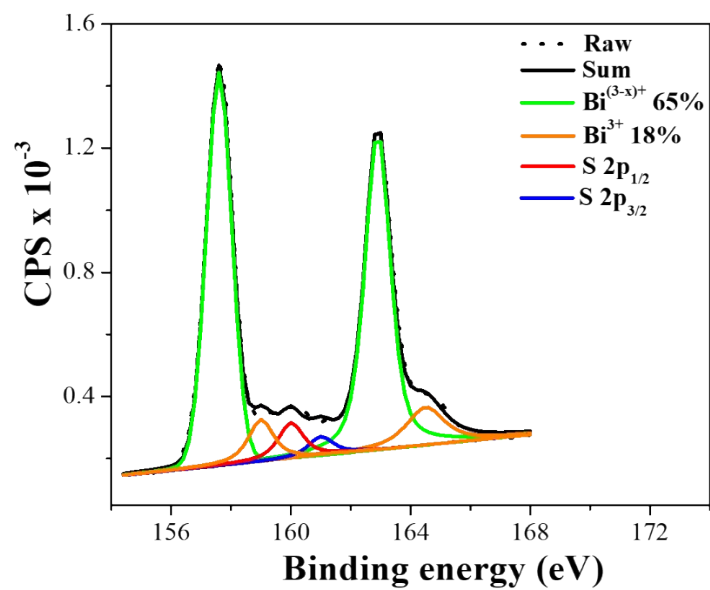


Fig. S4. Raw and deconvoluted Bi 4f and S 2p XPS spectra of BSA-Bi₂S₃ NPs.

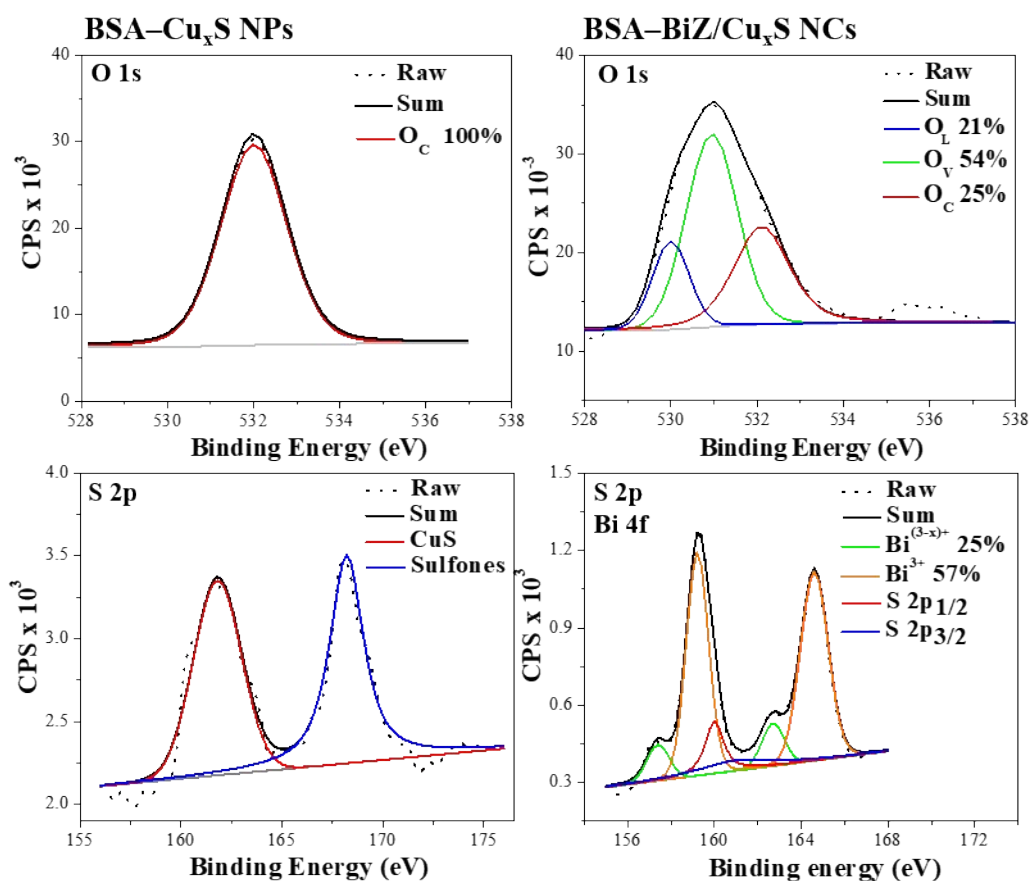


Fig. S5. Raw and deconvoluted XPS spectra of BSA-Cu_xS NPs and BSA-BiZ/Cu_xS NCs. The binding energies were corrected using C 1s (284.6 eV) as a standard. The lattice oxygen, chemisorbed or dissociated oxygen, oxygen vacancy, and defect are denoted as O_L, O_c, and O_V, respectively.

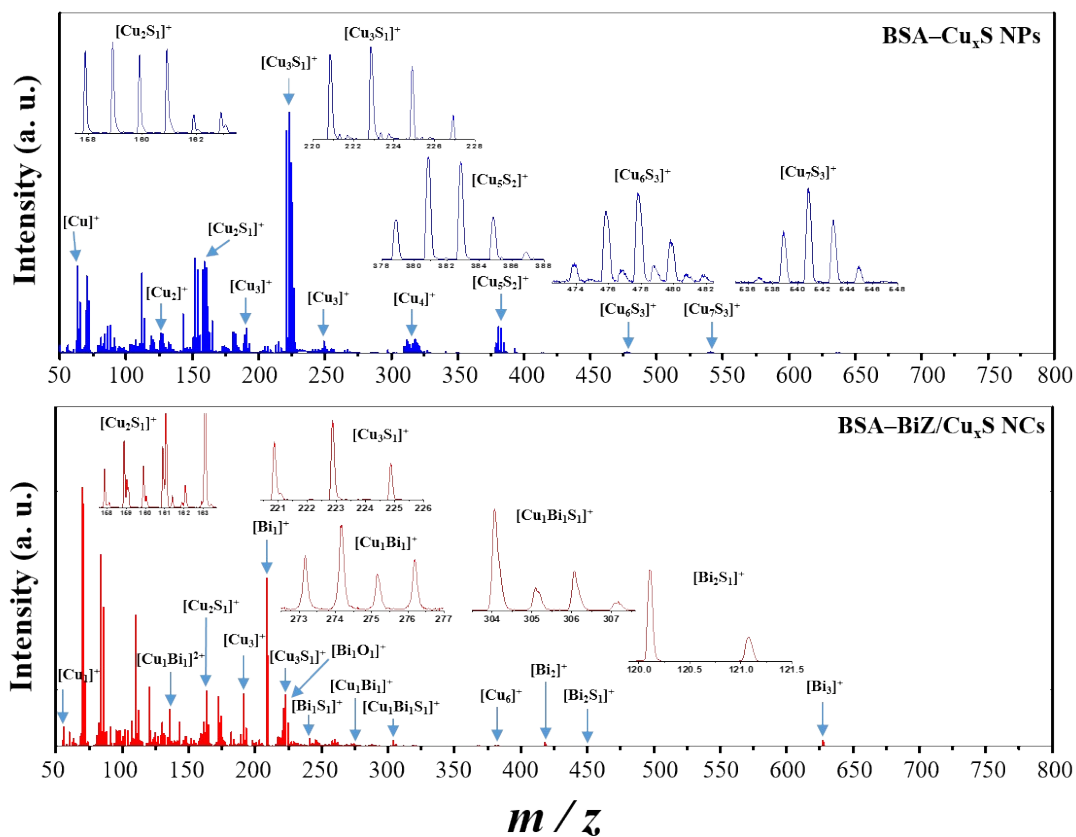


Fig. S6. LDI-MS spectra of BSA-BiZ/Cu_xS NCs and BSA-Cu_xS NPs. Insets show the theoretical isotope patterns. Peak intensities are plotted in arbitrary units (a. u.).

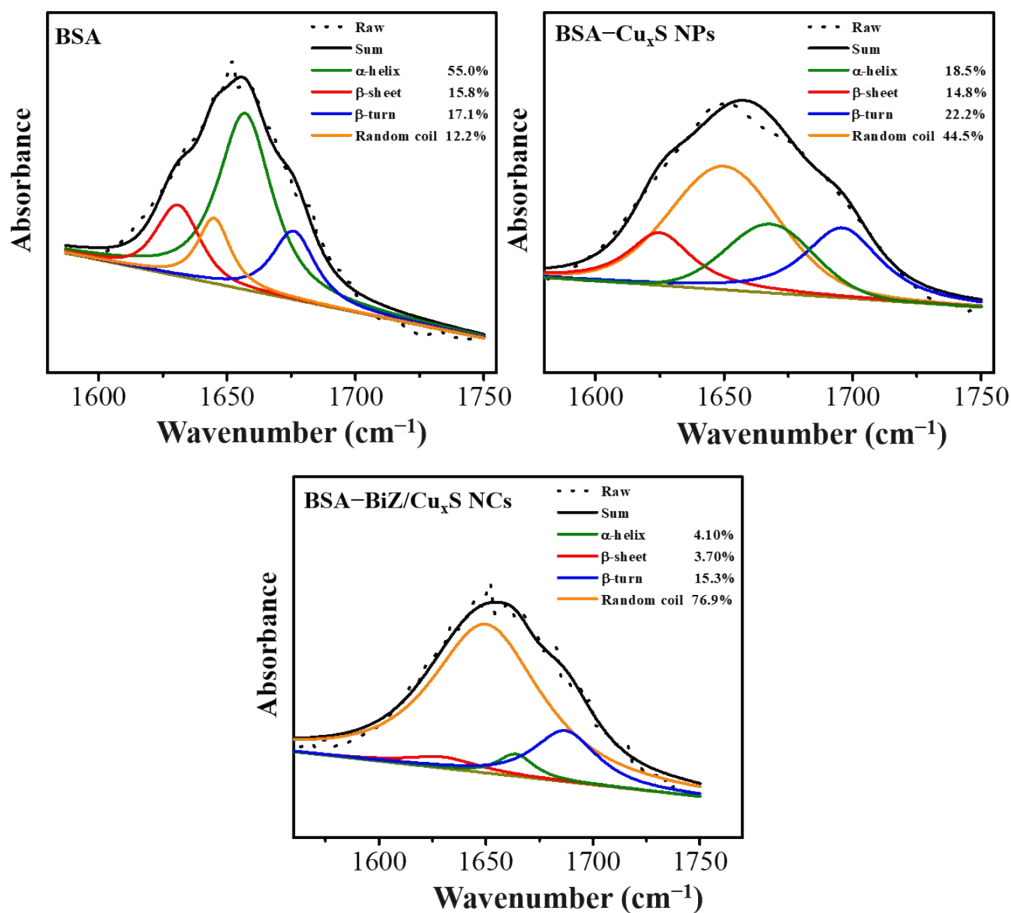


Fig. S7. Deconvoluted amide I band in the FT-IR spectra of BSA-BiZ/Cu_xS NCs, BSA-Cu_xS NPs, and free BSA. The concentration of BSA was the same (0.5 μM) in all four samples.

Deconvoluted FT-IR spectra of the amide I band (1600–1720 cm⁻¹) employed to study the compositions of α-helix (1643–1664 cm⁻¹), β-sheet (1615–1635 cm⁻¹), β-turn (1661–1687 cm⁻¹), and random coil (1632–1646 cm⁻¹). The deconvoluted amide I band displays much higher random coil structures in BSA-BiZ/Cu_xS NCs (76.9%) as compared to BSA-Cu_xS NPs (44.5%) and native BSA (12.2%), evidencing the higher partial denaturation of BSA after the formation of NCs in an alkaline solution.

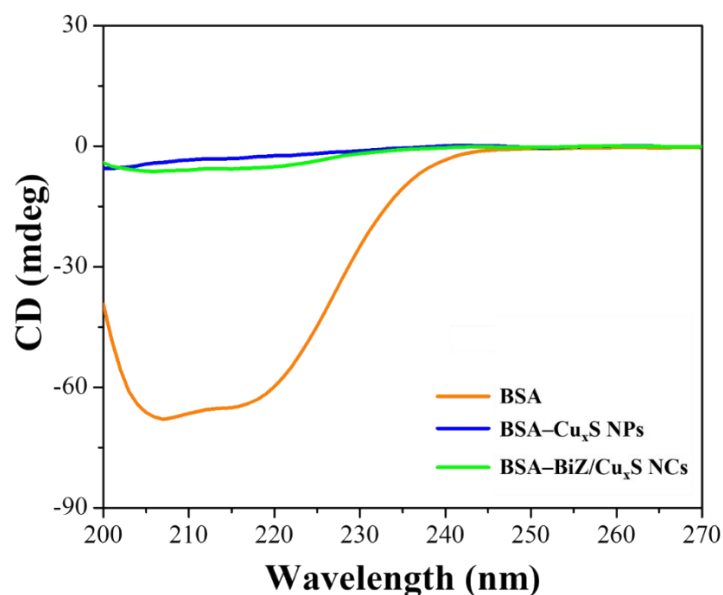


Fig. S8. CD spectra of BSA–BiZ/Cu_xS NCs, BSA–Cu_xS NPs, and free BSA dispersed in 10 mM sodium phosphate buffer (pH 7.4). The concentration of BSA was the same (0.5 μM) in all four samples. The percentage compositions of α -helix/ β -strands/random coil in the BSA–BiZ/Cu_xS NCs, BSA–Cu_xS NPs, and BSA analyzed by using K2D3 software available at <http://k2d3.orgic.ca> are 5%/19%/76%, 20%/36%/44%, and 57%/32%/11%, respectively.

The CD spectra of native BSA revealed the presence of α -helix (57%), β -strands (32%), and random coil (11%) structures are consistent with the FT-IR measurements. The secondary structures of BSA in the BSA–BiZ/Cu_xS NCs and BSA–Cu_xS NPs were faded to 5–20%, whereas the random coil structures increased to 44–76%, further corroborating the fact that BSA acted as a stabilizer/template to stabilize the nanomaterials.

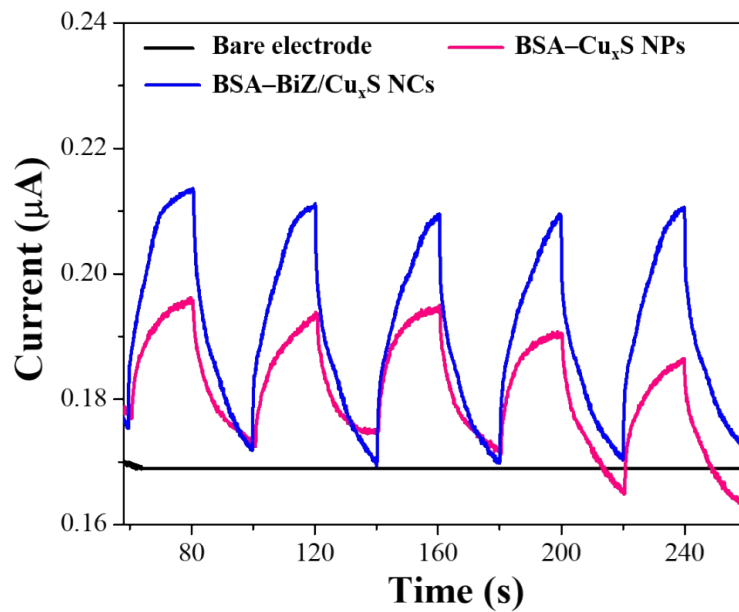


Fig. S9. Photocurrent response profile of BSA-BiZ/Cu_xS NCs and BSA-Cu_xS NPs in sodium sulfate solution (Na₂SO₄, 0.1 M) under white LED irradiation (0.31 W cm⁻²) at 0.25 V. The 'on' and 'off' time interval is 20 s.

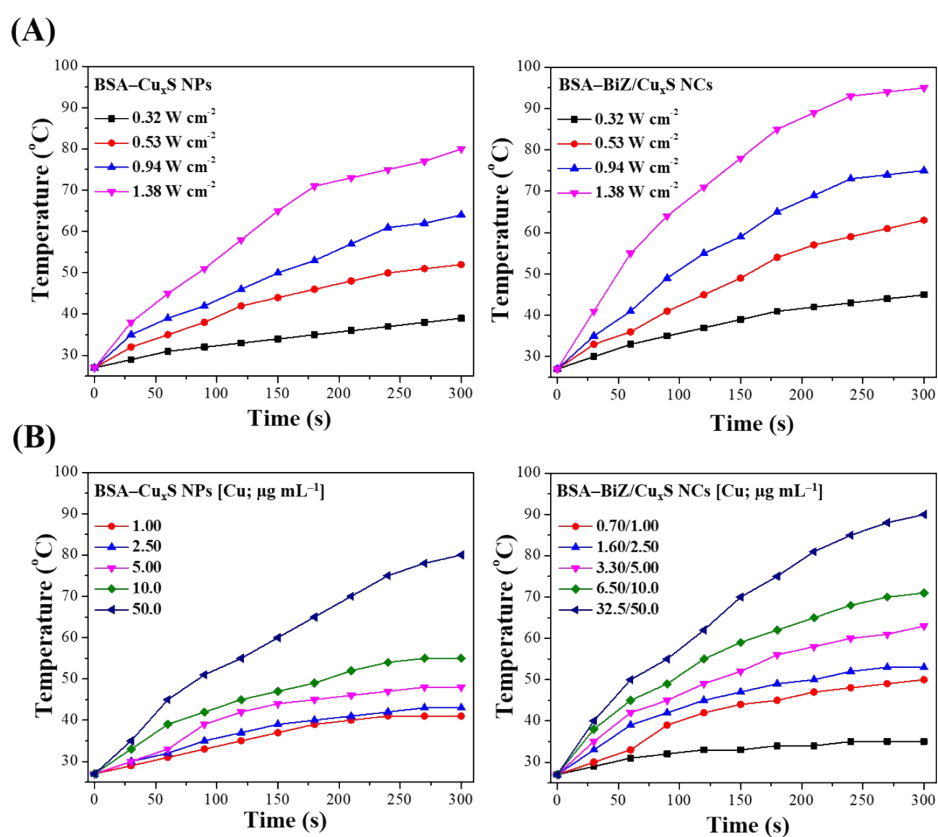


Fig. S10. (A) Power density- and (B) concentration-dependent temperature profile of BSA-BiZ/Cu_xS NCs and BSA-Cu_xS NPs in sodium phosphate buffer (10 mM, pH 7.4) solution. The concentration in (A) are 5.0 μg mL⁻¹ (in terms of Cu) and power density of the 808 nm NIR laser in (B) is 0.53 W cm⁻².

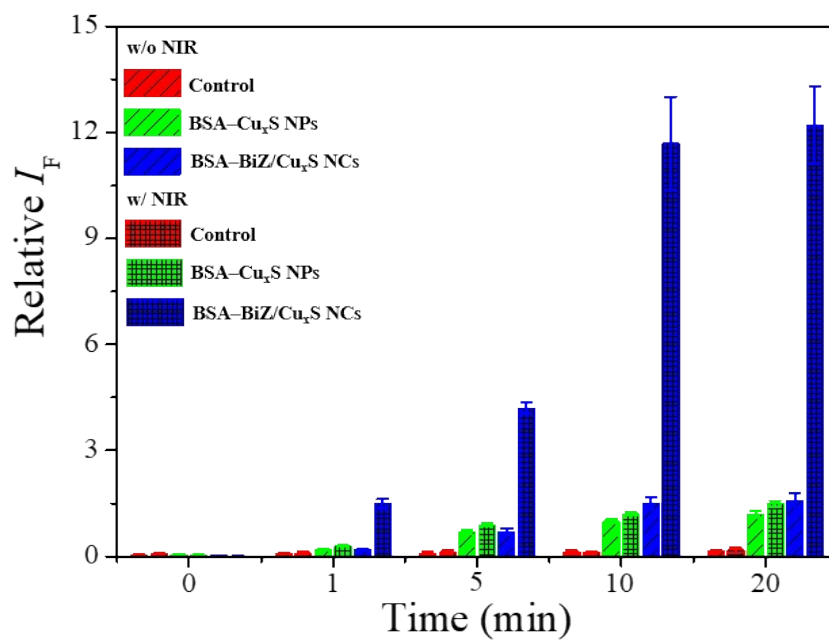


Fig. S11. Relative fluorescence intensity of AR (10 μ M) incubated separately with BSA-Cu_xS NPs and BSA-BiZ/Cu_xS NCs in the absence and presence of NIR irradiation for 1, 5, and 10 min. The error bars represent standard deviations derived from four repeated experiments. The concentration of NCs or NPs is 5.0 μ g mL⁻¹ (in terms of Cu).

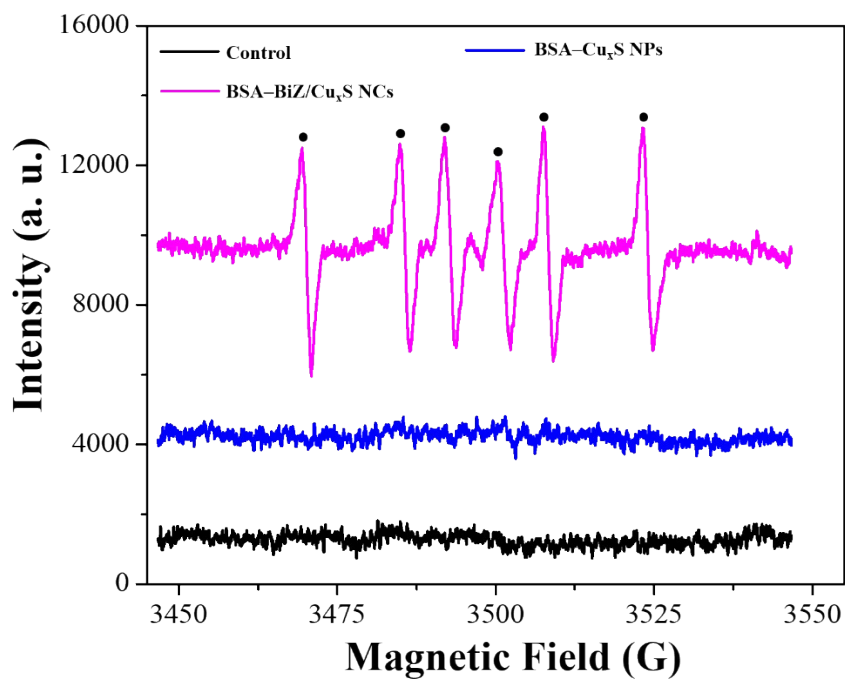


Fig. S12. ESR spectra of DMPO (10 mM) in sodium phosphate buffer (10 mM, pH 7.4) in the absence (as a control) and presence of $5.0 \mu\text{g mL}^{-1}$ (in terms of Cu) of BSA–BiZ/Cu_xS NCs and BSA–Cu_xS NPs without NIR laser irradiation. Black dots show the signal intensity from DMPO–OOH adducts ($a_N = 1.42 \text{ G}$ $a_H^\beta = 1.135 \text{ G}$).

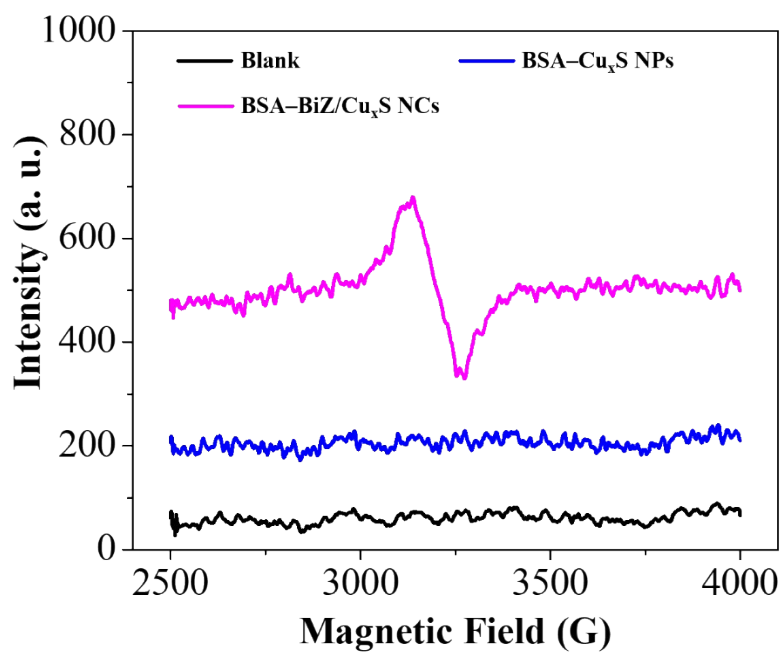


Fig. S13. ESR spectra of in the absence (as a control) and presence of $5.0 \mu\text{g mL}^{-1}$ (in terms of Cu) of BSA-Bi/Cu_xS NCs and BSA-Cu_xS NPs dispersed in sodium phosphate buffer (10 mM, pH 7.4).

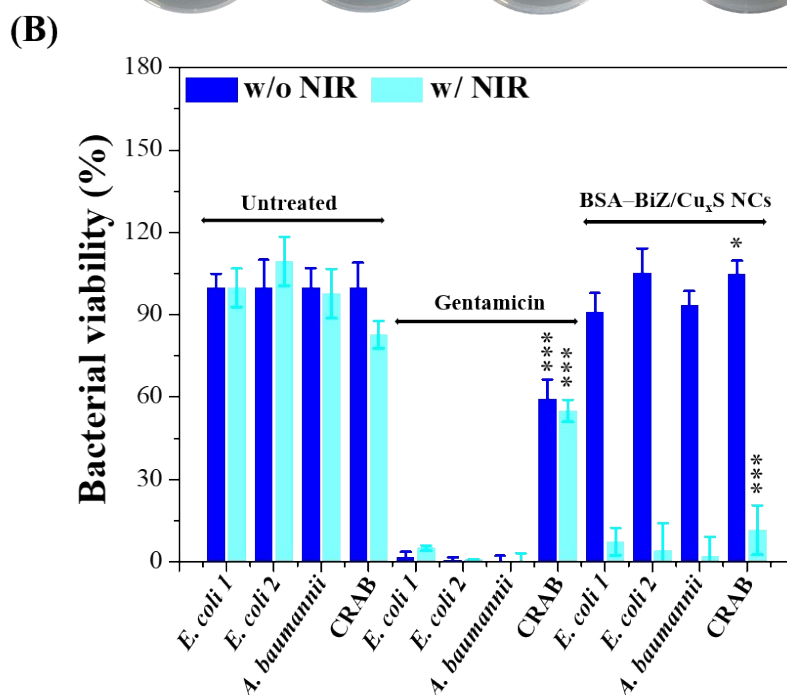
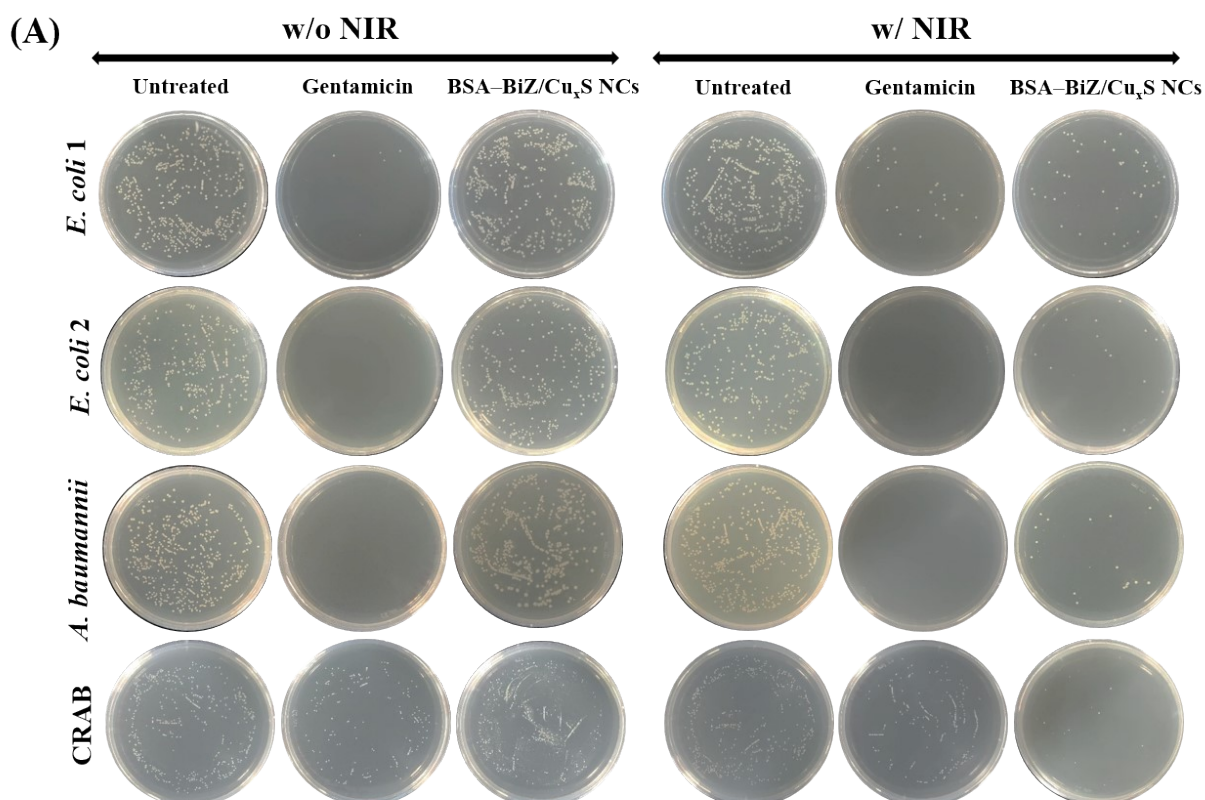


Fig. S14. (A) Representative colony formation and (B) bacterial viability of Gram-negative clinical isolates untreated and treated with gentamicin ($100 \mu\text{g mL}^{-1}$) or BSA-BiZ/Cu_xS NCs ($[\text{Cu}] = 4.0 \mu\text{g mL}^{-1}$) dispersed in 10 mM sodium phosphate buffer (pH 7.4) in absence and presence of NIR laser irradiation (808 nm, 0.53 W cm^{-2}) for 10 min. Error bars in (B), represent the standard deviation of three repeated measurements. Asterisks indicate statistically significant differences of the gentamicin and BSA-BiZ/Cu_xS NCs treated *S. epidermidis* groups as compared to the untreated ones w NIR and w/o NIR, respectively (* $p < 0.05$, ** $p < 0.005$, and *** $p < 0.001$).

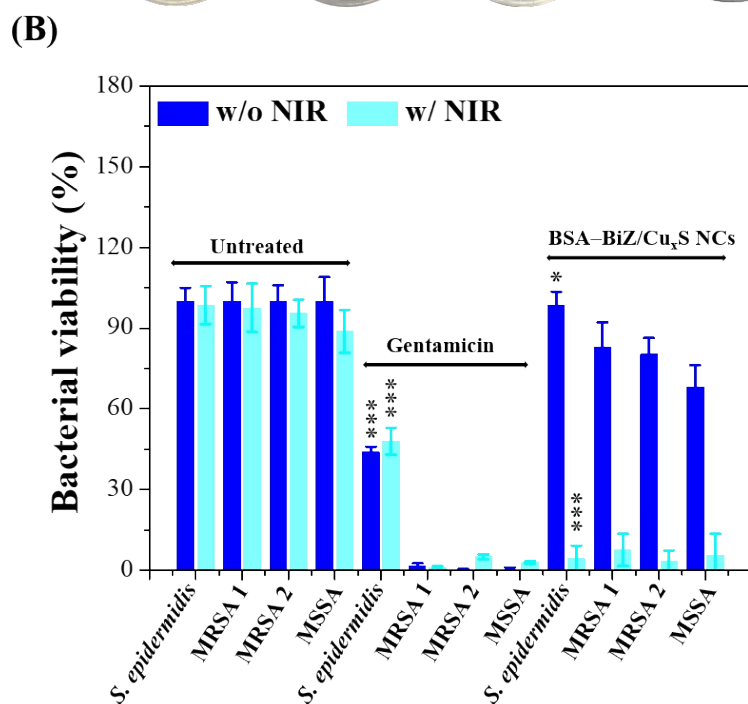
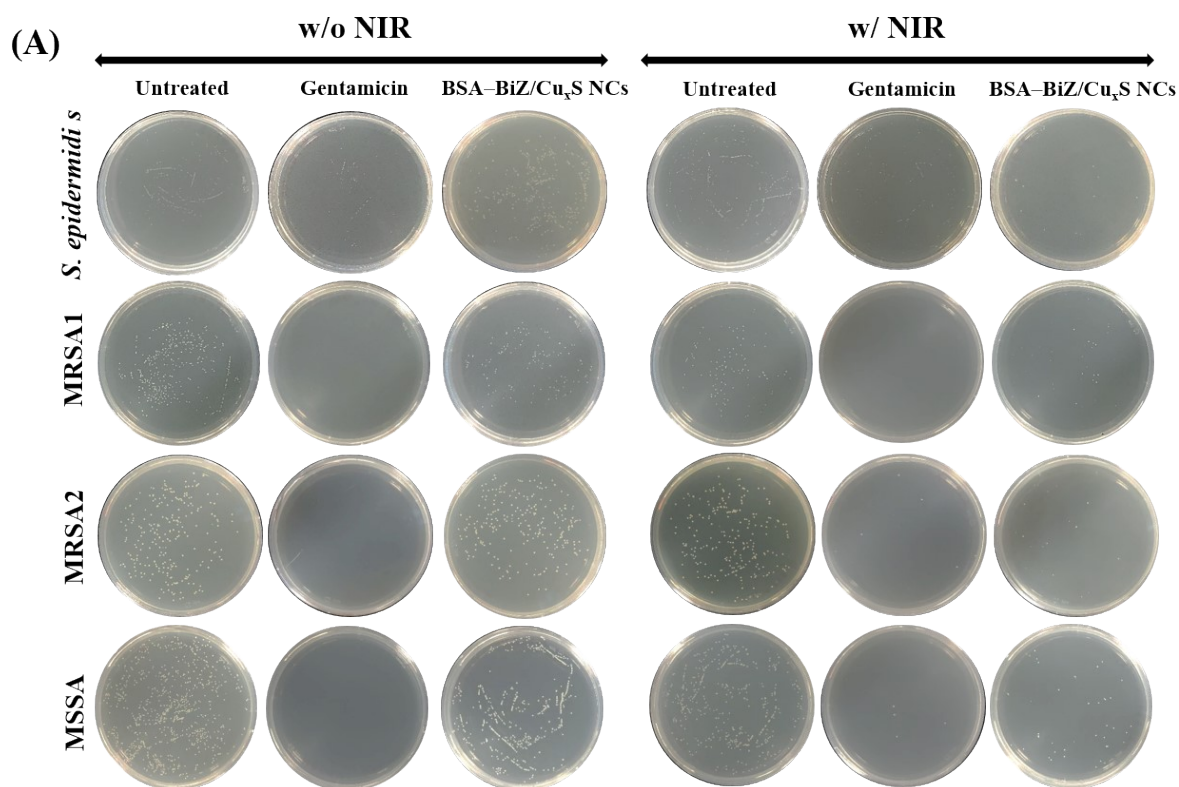


Fig. S15. (A) Representative colony formation and (B) bacterial viability of Gram-positive clinical isolates untreated and treated separately with gentamicin ($100 \mu\text{g mL}^{-1}$) and BSA-BiZ/Cu_xS NCs ($[\text{Cu}] = 4.0 \mu\text{g mL}^{-1}$) dispersed in 10 mM sodium phosphate buffer (pH 7.4) in absence and presence of NIR laser irradiation (808 nm, 0.53 W cm^{-2}) for 10 min. Error bars in (B), represent the standard deviation of three repeated measurements. Asterisks indicate statistically significant differences of the gentamicin and BSA-BiZ/Cu_xS NCs treated *S. epidermidis* groups as compared to the untreated ones w NIR and w/o NIR, respectively (* $p < 0.05$, ** $p < 0.005$, and *** $p < 0.001$).

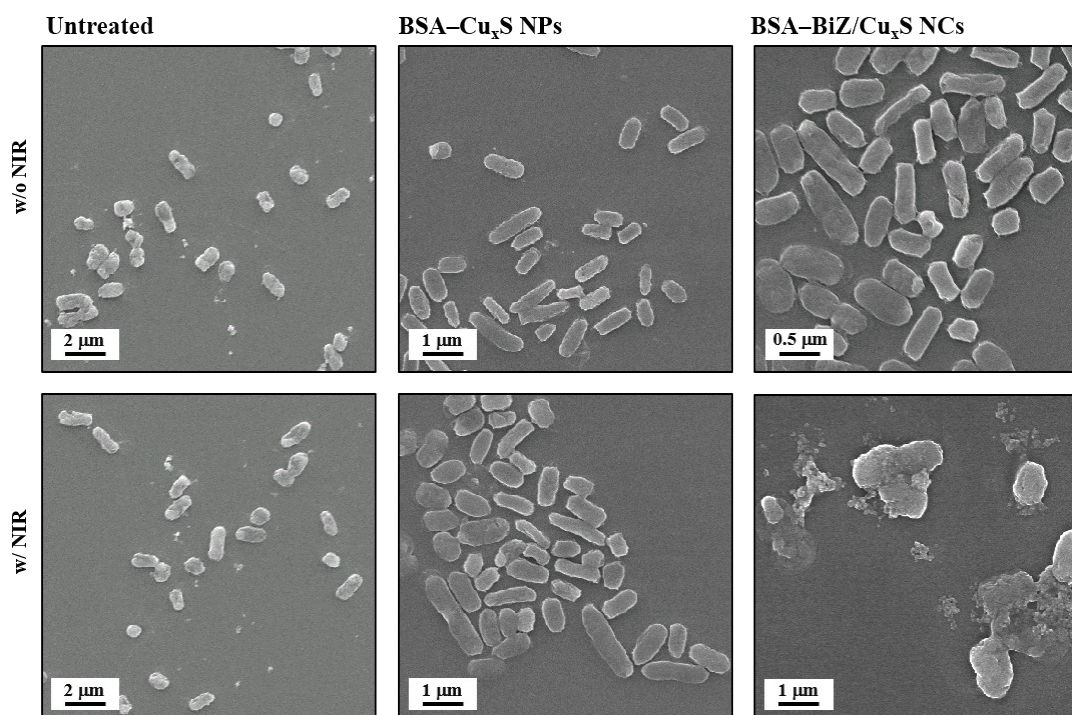


Fig. S16. SEM images of *E. coli* (1.0×10^4 CFU mL⁻¹) untreated and separately treated with $4.0 \mu\text{g mL}^{-1}$ (in terms of Cu) of BSA-BiZ/Cu_xS NCs and BSA-Cu_xS NPs without or with NIR laser irradiation (808 nm, 0.53 W cm^{-2}) for 10 min.

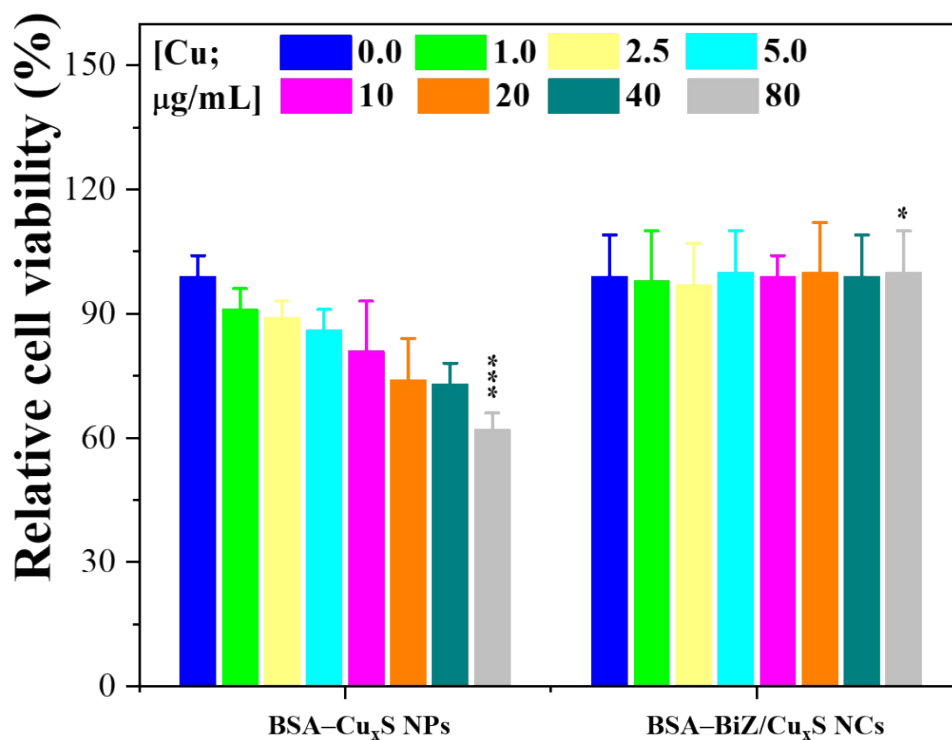


Fig. S17. Relative cell viability of HaCaT (1.0×10^4 cells well⁻¹) after separate incubation with various concentrations (in terms of Cu) of BSA-BiZ/Cu_xS NCs and BSA-Cu_xS NPs in DMEM containing 10% FBS for 48 h at 37 °C. The error bars represent the standard deviation of four repeated measurements. Asterisks indicate statistically significant differences of the BSA-Cu_xS NPs and BSA-BiZ/Cu_xS NCs treated groups (80 $\mu\text{g mL}^{-1}$) as compared to the untreated ones (0.0 $\mu\text{g mL}^{-1}$) (* $p < 0.05$, ** $p < 0.005$, and *** $p < 0.001$).

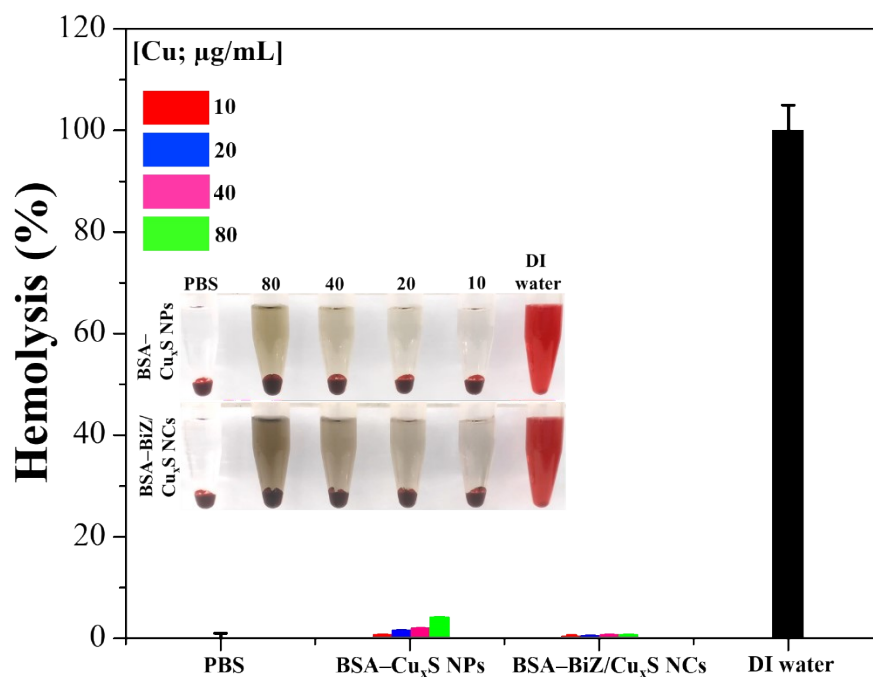


Fig. S18. Hemolytic activities of BSA-BiZ/Cu_xS NCs and BSA-Cu_xS NPs dispersed in PBS solution against RBCs. The RBCs treated with PBS and DI water served as negative and positive controls, respectively. Insets: photographs of RBCs (4% v/v) after incubation in PBS, DI water or various concentrations (in terms of Cu) of BSA-Bi/Cu_xS NCs or BSA-Cu_xS NPs. The error bars represent the standard deviation of four repeated measurements.

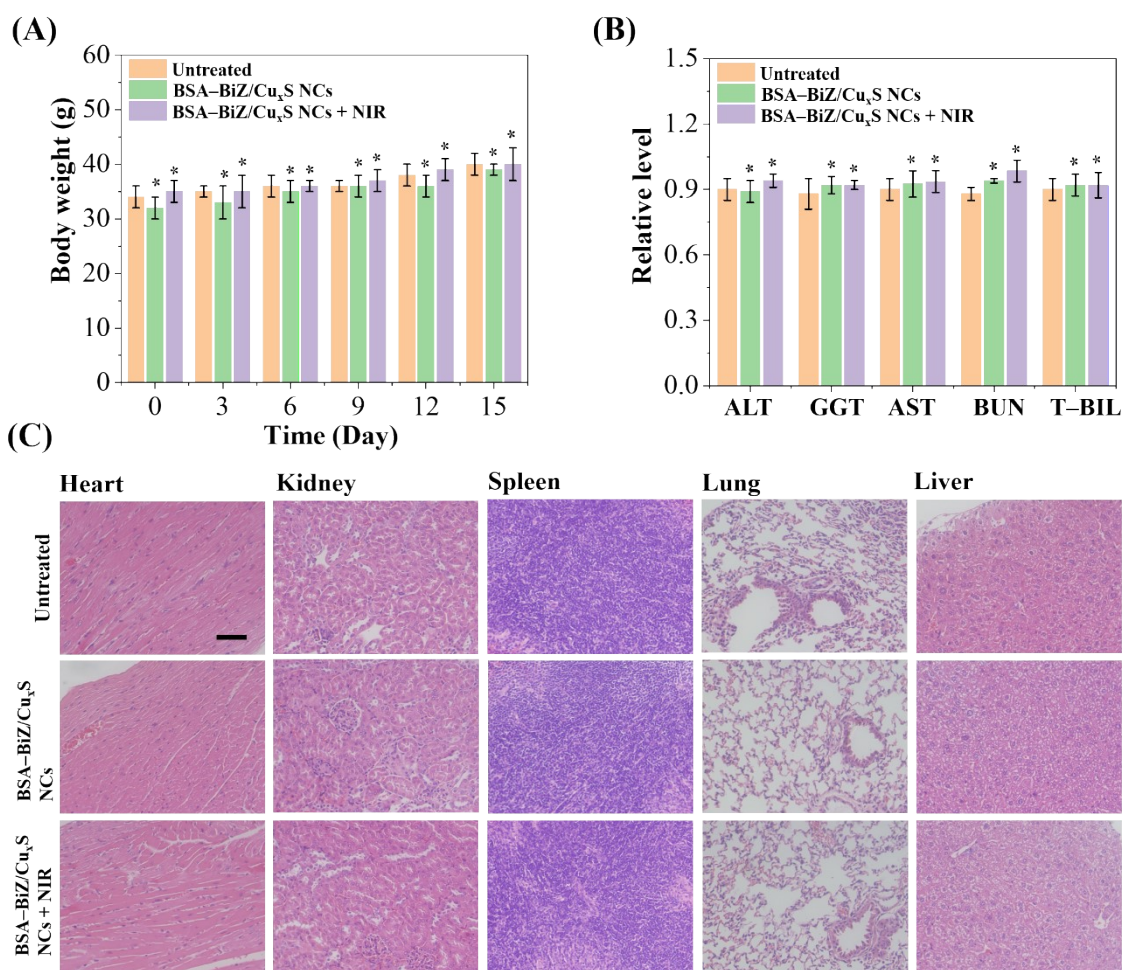


Fig. S19. (A) Body weight of untreated and treated mice with BSA-BiZ/Cu_xS NCs for 15 days. (B) Relative levels of ALT, GGT, AST, BUN, and T-BIL in the blood stream of mice before and after administration of BSA-BiZ/Cu_xS NCs for 15 days. (C) H&E staining of the tissues excised (thickness ~6 μm) at day 15 post treatment. Scale bar is 100 μm. Error bars in (A) and (B) show the standard deviation of three repeated measurements. Asterisks indicate statistically significant differences of the BSA-BiZ/Cu_xS NCs treated groups (w/ NIR and w/o NIR) as compared to the untreated ones (*p < 0.05, **p < 0.005, and ***p < 0.001).

Table S1. Atomic compositions of BSA–BiZ/Cu_xS NCs, BSA–Cu_xS NPs, and BSA–Bi₂S₃ NPs estimated by elemental analysis and ICP-MS.

Nanomaterials	O (%) ^a	S (%) ^a	Cu (%) ^a	Bi (%) ^a
BSA–BiZ/Cu _x S NCs	17.5±0.05	23.3±0.06	26.7±1.10	14.2±0.06
BSA–Cu _x S NPs	19±1.81	16.7±0.83	52.6±1.5	ND ^b
BSA–Bi ₂ S ₃ NPs	26.4±0.13	7.0±0.05	ND ^b	16.1±0.12

^aerror bars represent standard deviations from four repeated experiments; ^bnot detected

Reference:

- 1 Q. Guo, F. Liang, Z. Sun, Y. Wang, X. B. Li, S. G. Xia, Z. C. Zhang, L. Huang, L. Z. Wu, *J. Mater. Chem. A*, 2020, **8**, 22601.