Supplementary Information

S-nitrosylated Au@COF nanohybrids for synergistic light-nitric oxide killing of bacteria

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Experimental

Materials

Cetyltrimethylammonium bromide (CTAB, 99%), Griess reagent (modified), and ethanedithiol were purchased from Sigma-Aldrich. 2-methylpropionitrile (AIBN), dihydroethidium (DHE), and L-tyrosine were purchased from Aladdin. Silver nitrate ($AgNO_3$, 99%), chloroauric acid ($HAuCl_4 \cdot 4H_2O$), hydrochloric acid (HCl, 36-38%), ascorbic acid (AA, 99.7%), sodium borohydride ($NaBH_4$), acetic acid (CH_3COOH,

36%), acetone, toluene, methanol, and sodium nitrite ($NaNO_2$, 99%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Sodium oleate (NaOL, 98%) was purchased from TCI. NH_2-PEG5000-SH and NH_2-PEG5000 were purchased from Pengsheng Biotechnology. 1,3,5-Tris(4-aminophenyl)benzene (TPB) and 2,5-divinyl terephthalaldehyde (DVA) were purchased from Jilin Zhongke Yanshen Technology Co., Ltd. The bacterial strains, Gram-negative *Staphylococcus aureus* (*S. aureus*, ATCC-14458) and Gram-positive *Escherichia coli* (*E. coli*, ATCC-8739), were obtained from Luwei Microbial Technology Co., Ltd. (Shanghai, China).

Synthesis of AuNRs

AuNRs were prepared according to previously published method.[1] The synthesis of AuNRs employed a binary surfactant mixture composed of CTAB and sodium oleate to grow Au seeds into NRs. Briefly, the seed solution was prepared as follows: 5 mL of 0.5 mM $HAuCl_4$ was mixed with 5 mL of 0.2 M CTAB solution in a 20 mL scintillation vial. 0.6 mL of fresh 0.01 M $NaBH_4$ was diluted to 1 mL with water and injected into the Au(III)-CTAB solution under vigorous stirring (1,200 rpm). The color of the solution changed from yellow to brownish yellow, and the stirring was stopped after 2 min. The seed solution was aged at room temperature for 30 min prior to further use. In order to synthesize the target AuNRs, a growth solution was prepared by dissolving 3.6 g CTAB and 0.496 g NaOL in 100 mL warm water (~50 °C) in a 250 ml Erlenmeyer flask. The solution was allowed to cool down to 30 °C, and then 9.6 mL of 4 mM $AgNO_3$ solution was added. The mixture was kept undisturbed at 30 °C for 15 min, after which 100 mL of 1 mM $HAuCl_4$ solution was added. The solution became colorless after 90 min of stirring (700 rpm), and then 0.84 mL of HCl (37 wt. % in water, 12.1 M) was introduced to adjust the pH and acidify the solution. After 15 min of slow stirring at 400 rpm, 0.5 mL of 0.064 M ascorbic acid was added and the solution was vigorously stirred for 30 s. Finally, 80 μL of seed solution was injected into the growth solution. The resultant mixture was stirred for

30 s and left undisturbed at 30 °C for 12 h to complete NR growth. The products were isolated by centrifugation at 7,000 rpm for 30 min and washed with DI water. Finally, the obtained AuNRs were dispersed in 90 mL of H_2O.

Synthesis of AuNR@COFs

For the synthesis of core-shell AuNR@COFs, a two-step process was used.[2] Firstly, NH_2-PEG5000-SH (20 mg) was added to 20 mL of AuNRs dispersion and stirred overnight at room temperature. After this incubation step, the dispersion was centrifuged at 10,000 rpm for 10 min and the precipitate was washed with ultrapure water. The obtained amine-modified AuNR cores were then dispersed in 20 mL of methanol. DVA and TPB were dissolved in acetone to prepare 25 mM and 30 mM precursor solutions, respectively. Then, 500 μL DVA and 500 μL TPB solution were added into AuNR dispersion, followed by adding acetic acid (200 μL). The mixed solution was vortexed for 10 seconds and left to stand for 1 h. After that, NH_2-PEG5000 (40 mg) was added into the solution and left undisturbed overnight. After the reaction was complete, the mixture was centrifuged and washed with ethanol twice. The final precipitate was re-suspended in 4 mL of toluene.

Synthesis of AuNR@COFs-SH

4 mL of AuNR@COFs toluene dispersion and AIBN (80 mg) were added to a 25 mL round-bottom flask under a N_2 atmosphere. Then, 1,2-ethanedithiol (500 μL) was injected into the above system under vigorous stirring. After 24 h of reaction at 80 °C, the mixture was centrifuged and the obtained AuNR@COFs-SH were washed three times with toluene and acetone.

Synthesis of AuNR@COFs-SNO

15 mL of $NaNO_2$ solution (pH = 3.5, 6 mg/mL) was mixed with AuNR@COFs-SH at 0 °C in the dark and stirred for 2 h. After the reaction, the mixture was centrifuged at 8,000 rpm for 10 min and washed 3 times with DI water. Finally, the AuNRs@COFs-

SNO was stored away from light at 4℃.

Characterizations

The morphology of nanoparticles was examined using a transmission electron microscope (TEM, Hitachi HT7700). Fourier-transform infrared (FTIR) spectra were obtained using a Nicolet iN10 FTIR spectrometer (Nicolet Instrument Co.). Energy-dispersive X-ray spectroscopy (EDS) elemental mapping analysis was performed using a field-emission transmission electron microscope (Tecnai G2 F20 S-TWIN). Bacteria samples were examined using a scanning electron microscope (SEM, Quanta 250FEG) at an operating voltage of 10 kV. Extinction spectra were acquired using a UV-vis-NIR spectrometer (Shimadzu UV-3600 Plus). Fluorescence spectra were obtained using a fluorescence spectrophotometer (F-4600 FL Spectrophotometer). Zeta potential and dynamic light scattering (DLS) measurements were conducted using a Malvern Zetasizer Nano ZS. N_2 adsorption−desorption isotherms were measured with a Micromeritics ASAP 2460 automatic volumetric instrument. The samples were degassed at 150 °C for 8 h under vacuum before analysis. The surface areas were calculated from the adsorption data based on the Brunauer−Emmett−Teller (BET) model. The pore size distribution of COFs was evaluated by nonlocalized density functional theory (NLDFT).

Photothermal Property Evaluation

To evaluate the photothermal performance of AuNR@COFs-SNO, dispersions with different material concentrations (50, 75, 100, and 150 μg/mL) were irradiated with a 1064 nm laser at 1 W/cm^2 for 10 minutes. The real-time temperature changes of the dispersions under laser irradiation were monitored using a digital thermometer. The thermal cooling curve of AuNR@COFs-SNO was recorded after the laser was turned off. The photothermal conversion efficiency (η) was calculated using the following equations:[3]

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_0}{I(1 - 10^{-A_{1064}})} \quad (1)$$

$$\tau_s = \frac{m_d C_d}{hS} \quad (2)$$

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

$$Q_0 = hS(T_{max,water} - T_{surr}) \quad (4)$$

Where:

h is the heat transfer coefficient,

S is the material surface area,

A is the surface area of the container,

T_{max} is the maximum temperature reached during laser irradiation,

T_{surr} is the ambient temperature,

Q_0 is the heat dissipated from the system,

I is the laser power density,

A_{1064} is the absorbance of the solution at the laser wavelength (1064 nm),

The value of τ_s can be figured out from the fitting curve of the thermal cooling curve. Equation (2) can be used to the value of hS. Then, Q_0 is obtained via Equation (4).

NO Detection

The amount of NO released from AuNR@COFs-SNO was measured using Griess reagent. For the detection of NIR controlled NO release, AuNR@COFs-SNO was dispersed in water at 100 μg/mL and irradiated with a 1064 nm laser (1 W/cm²) for 30 minutes. At various time points (5, 10, 15, 20, and 30 minutes), aliquots of the reaction dispersion were collected and mixed with an equal volume of Griess reagent. The mixture was then centrifuged, and the absorbance (at 540 nm) of the supernatant was measured using a UV-vis-NIR spectrometer. The quantification of NO release over time was then obtained by correlating the absorbance values with an established standard curve using $NaNO_2$ solution of known concentrations. To determine the

amount of loaded RSNO, a dispersion of AuNR@COFs-SNO (100 μg/mL) was incubated in a water bath at 80 °C for 1 hour. After the dispersion was cooled to room temperature, an equal volume of Griess reagent was added. Then, the mixture was centrifuged, and the absorbance of the supernatant at 540 nm was measured to quantify the RSNO loading capacity based on a pre-established calibration curve.

Superoxide Anion Detection

The release of superoxide anions from different samples under light and dark conditions was detected using dihydroethidium (DHE). First, a 40 μM DHE working solution was prepared. The sample dispersions were incubated under light and dark conditions for 10 minutes, mixed with an equal volume of the DHE working solution, and centrifuged to collect the supernatant. The fluorescence of the supernatant was measured at an excitation wavelength of 490 nm. The entire experiment was conducted under light-protected conditions.

Qualitative Determination of $ONOO^-$

L-tyrosine was employed as a probe molecule for $ONOO^-$ generated in a weakly alkaline solution. The dimerization of L-tyrosine would be produced through the oxidation of $ONOO^-$. In a typical experiment, a 10 mL aqueous solution containing PBS (0.10 M, pH 8.2), $NaHCO_3$ (0.015 M), and L-tyrosine (0.5 μM) was prepared. Subsequently, AuNR@COFs-SNO was added to the solution, which was then irradiated with or without a 1064 nm laser for 10 minutes. Following this, $ONOO^-$ was detected using a fluorescence spectrometer at an excitation wavelength of 313 nm.

In Vitro Antibacterial Tests:

To assess the antimicrobial efficacy of AuNR@COFs-SNO, *Escherichia coli* and *Staphylococcus aureus* were selected as model bacteria. Ten independent parallel experimental groups were established: (1) Control (-); (2) COFs (-); (3) AuNR@COFs (-); (4) COFs-SNO (-); (5) AuNR@COFs-SNO (-); (6) Control (+); (7)

COFs (+); (8) AuNR@COFs (+); (9) COFs-SNO (+); (10) AuNR@COFs-SNO (+), where (+) denotes with NIR laser irradiation, while (-) means without. A 100 μL aliquot of bacterial suspension (0.9% physiological saline) in the logarithmic growth phase (10^6 CFU/mL) was mixed with an equal volume of the nanoparticle dispersion and the mixture was added to the wells of a microplate. Groups (1) to (5) were incubated in the dark for 10 minutes, while groups (6) to (10) were incubated under NIR irradiation (1064 nm, 1 W/cm²) for 10 minutes. Subsequently, 100 μL of the mixture was spread onto agar plates, which were then incubated at 37°C for 16 hours. After incubation, the bacterial colonies on the agar plates were counted to determine the corresponding bacterial survival rates.

Determination of Half-Maximal Inhibitory Concentration (IC50):

Gradient concentrations of AuNR@COFs-SNO dispersions (0, 20, 40, 60, 80 and 100 μg/mL) were mixed with bacterial cultures in the logarithmic growth phase. Following 10 minutes irradiation with a 1064 nm laser, the antibacterial rate was determined using the plate colony counting method. The IC50 value, corresponding to the material concentration required to achieve a 50% inhibition rate, was calculated by fitting the concentration-inhibition rate relationship curve.

In Vitro Antibiofilm Experiments:

A 100 μL aliquot of bacterial culture in the logarithmic phase (5×10^7 CFU/mL) was added to each well of a microplate and incubated at 37°C for 48 hours under static conditions to facilitate biofilm formation. Subsequently, the excess culture medium and planktonic bacteria were removed. To evaluate the anti-biofilm activity of nanoparticles, ten independent parallel experimental groups were established: (1) Control (-); (2) COFs (-); (3) AuNR@COFs (-); (4) COFs-SNO (-); (5) AuNR@COFs-SNO (-); (6) Control (+); (7) COFs (+); (8) AuNR@COFs (+); (9) COFs-SNO (+); (10) AuNR@COFs-SNO (+), where (+) indicates near-infrared (NIR)

laser irradiation, while (-) means without. 100 μL of nanoparticle dispersion was added to the well. After the corresponding treatments, the cultures were further incubated statically for another 48 hours. The medium, planktonic bacteria, and nanoparticles were then washed away with PBS for three times. The biofilms were fixed by adding methanol. Next, 100 μL of 1% crystal violet solution was added to each well to stain the biofilms for 30 minutes. After staining, excess dye was removed by washing with PBS, and the wells were air-dried at room temperature. Photographs were taken to visualize the biofilm coloration in each experimental group. Finally, absolute ethanol was added to the wells and incubated for 30 minutes, followed by UV-vis spectrophotometric measurement of the optical density at 550 nm (OD_{550}) to quantify the biofilm formation.

Supporting Figures

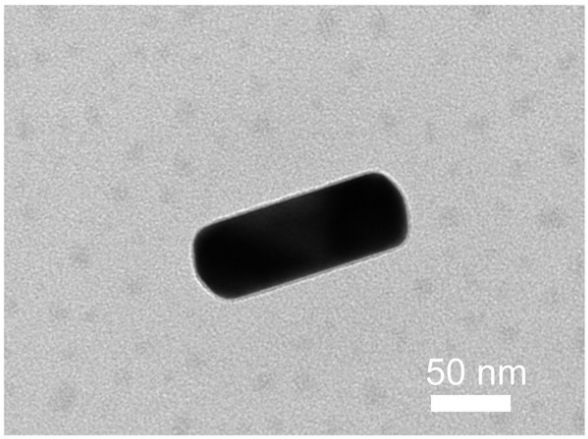

Figure S1. TEM image of AuNR@PEG.

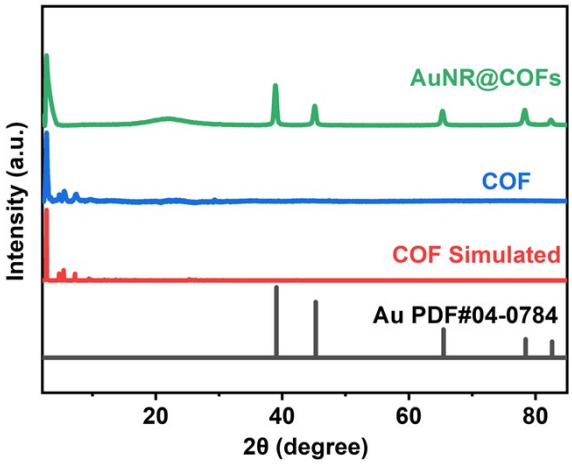

Figure S2. Powder XRD patterns for the COF (blue) and AuNR@COFs (green).

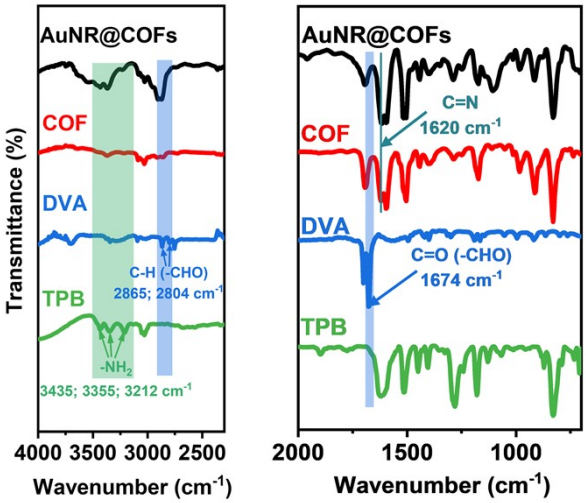

Figure S3. FTIR spectra of TPB (green), DVA (blue), the COF (red), and AuNR@COFs (black).

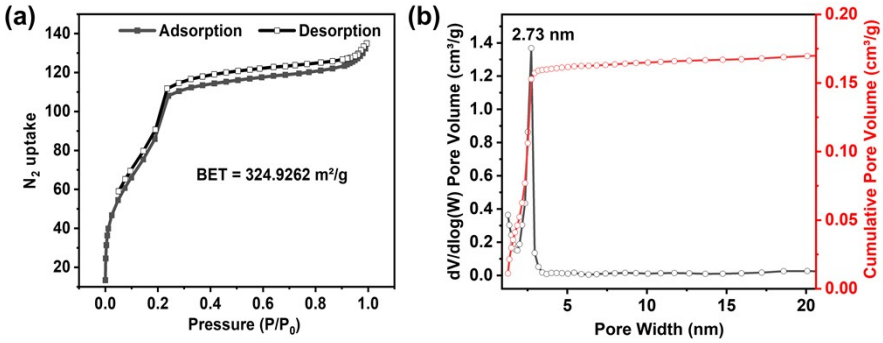

Figure S4. (a) N_2 adsorption−desorption isotherms; (b) Pore size distribution (black dots) and cumulative pore volume (red dots) profiles of the COFs. Pore size distributions of the COFs were calculated by using the NLDFT model.

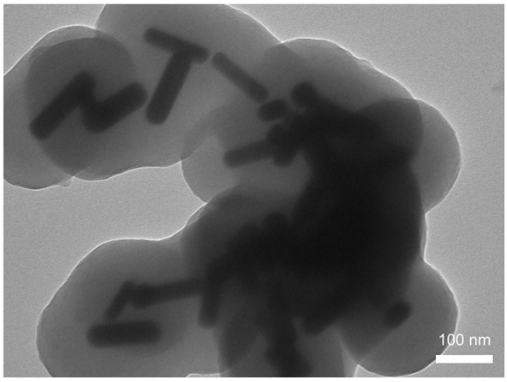

Figure S5. TEM image of AuNR@COFs-SH synthesized without PEG pre-treatment.

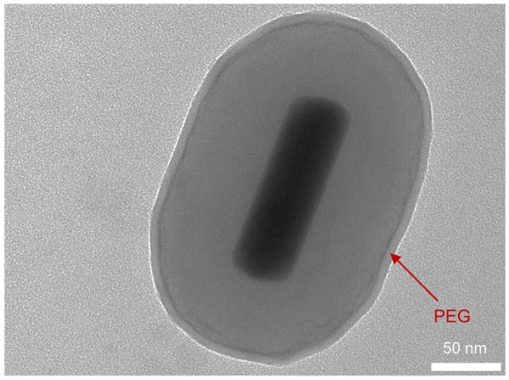

Figure S6. TEM image of AuNR@COFs-PEG.

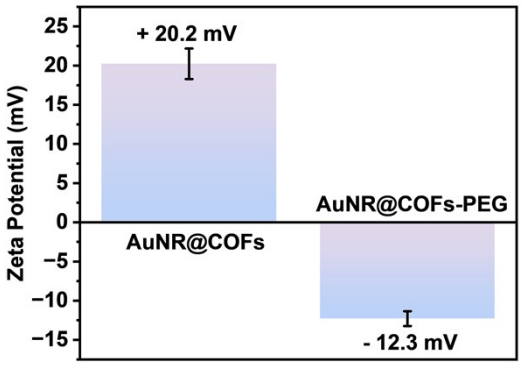

Figure S7. Zeta potential of AuNR@COFs and AuNR@COFs-PEG.

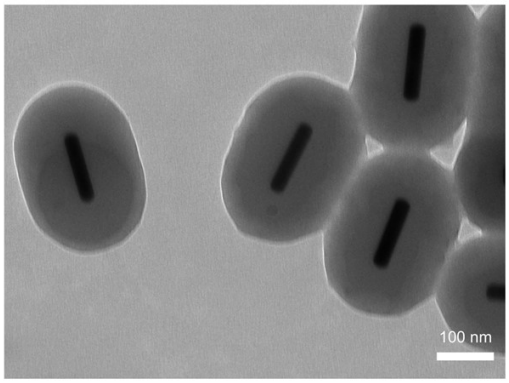

Figure S8. TEM image of AuNR@COFs-SH (with PEG modification).

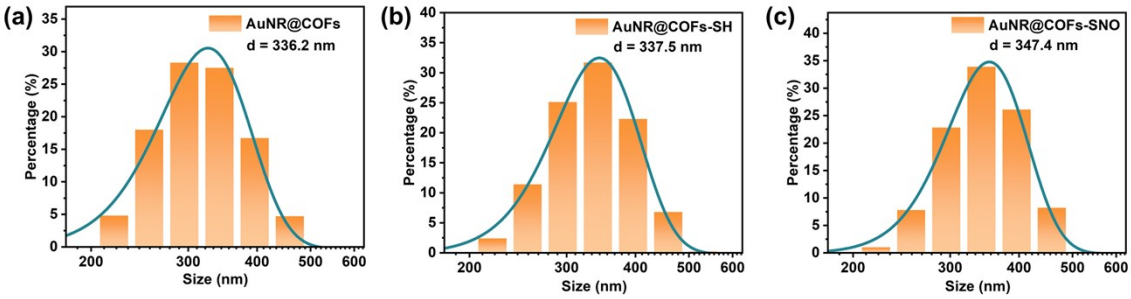

Figure S9. Size histogram of AuNR@COFs (a), AuNR@COFs-SH (b) and AuNR@COFs-SNO (c).

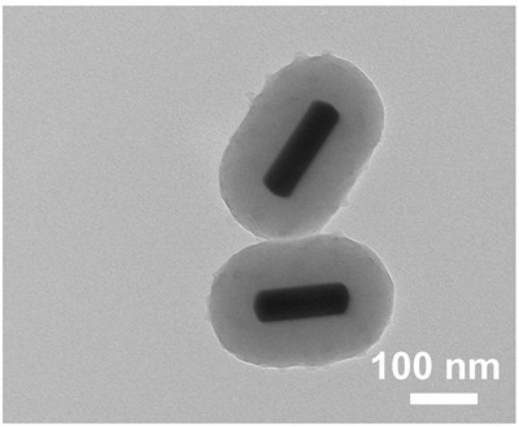

Figure S10. TEM image of AuNR@COFs-SNO after immersion in PBS solution (pH 5.5) for 24 h.

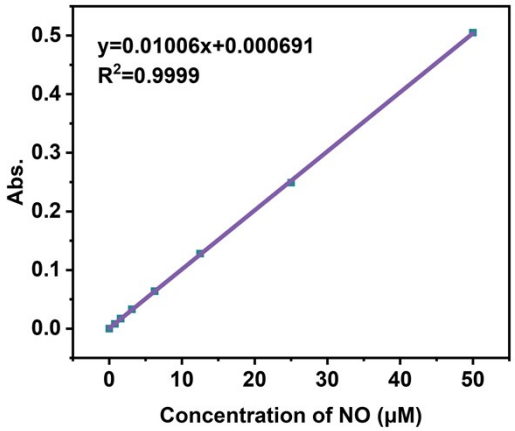

Figure S11. The standard calibration curve for NO release determination (linear regression correlation coefficients $R^2 = 0.9999$).

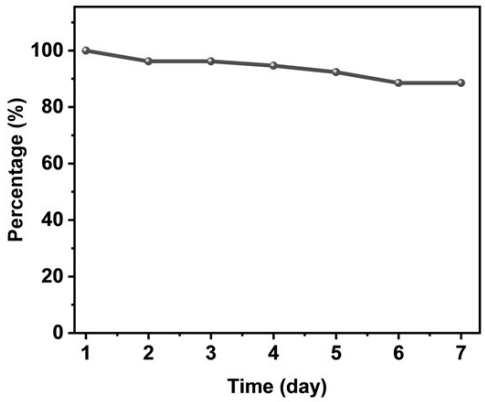

Figure S12. The nitric oxide store stability of AuNR@COFs-SNO within 7 days at dark, low temperature (-20 ℃) condition.

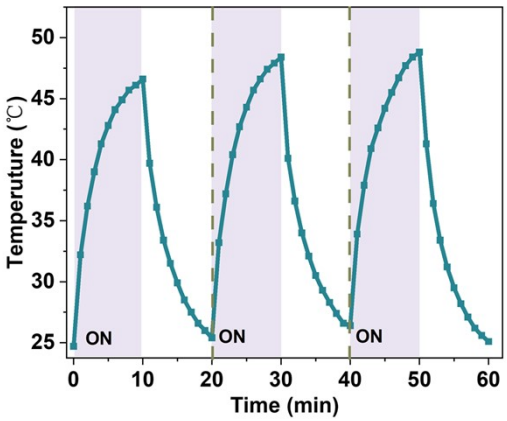

Figure S13. AuNR@COFs-SNO solution temperature changes under three irradiation on/off cycles (1064 nm, 1 W/cm²).

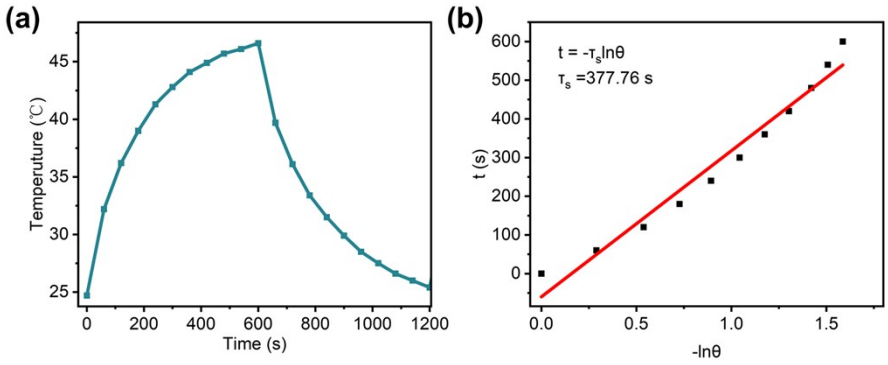

Figure S14. (a) Heating-up and cooling curve of AuNR@COFs-SNO. (b) Plot of cooling time versus negative natural logarithm of driving force temperature obtained from the cooling curve, and the linear fitting result.

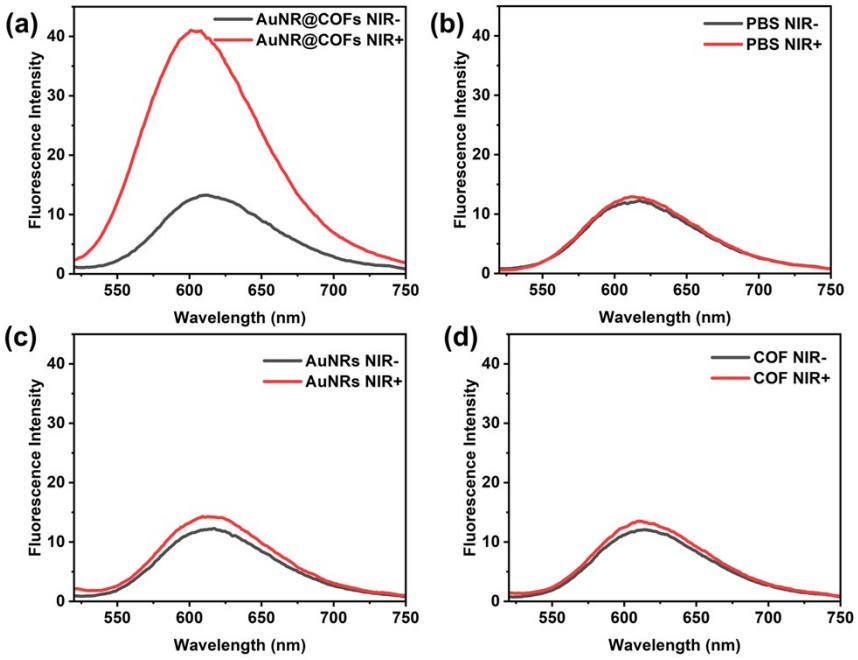

Figure S15. Fluorescence spectra for $O_2^{\cdot-}$ detection from different solution samples in the presence of dihydroethidium under NIR irradiation and dark conditions: (a) AuNR@COFs, (b) PBS, (c) AuNRs, and (d) COF.

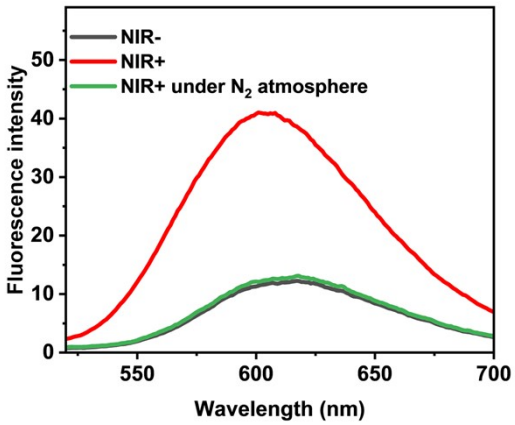

Figure S16. Fluorescence spectra for $O_2^{\cdot-}$ detection from AuNR@COFs with dihydroethidium under N_2 atmosphere.

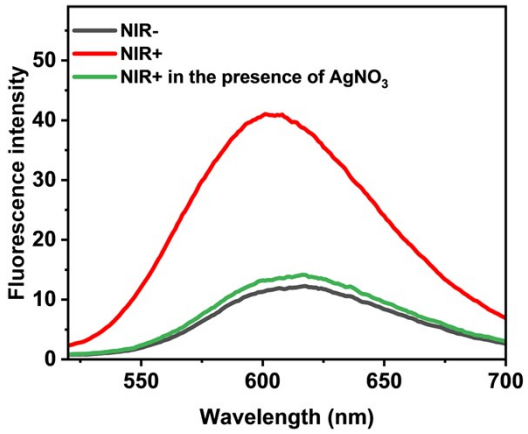

Figure S17. Fluorescence spectra for $O_2^{\cdot-}$ detection from AuNR@COFs with dihydroethidium in the presence of $AgNO_3$ as an electron scavenger.

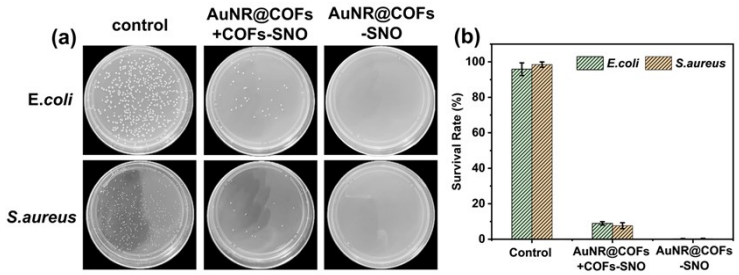

Figure S18. Typical photographs of *E. coli* and *S. aureus* colonies (a) and the calculated statistics of bacterial survival (b) after treatment with AuNR@COFs and COFs-SNO under NIR irradiation sequentially for comparison.

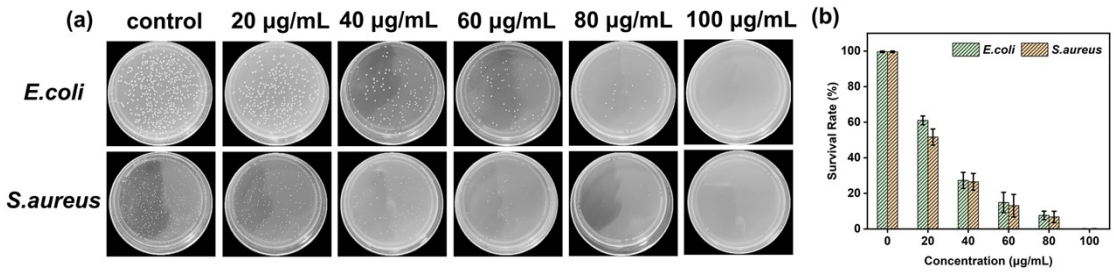

Figure S19. Typical photographs of *E. coli* and *S. aureus* colonies (a) and the calculated statistics of bacterial survival (b) after treatment with AuNR@COFs-SNO of different concentrations under NIR irradiation.

References

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