Supporting information for

Porphyrin covalent organic nanodisks synthesized using acid-assisted exfoliation for improved bactericidal efficacy

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Table S1. Averaged thickness and sizes for DhaTph, N-CONs and A-CONs measured by AFM.

	Size (nm)	Thickness (nm)
DhaTph	123.4 ± 35.7	13.5 ± 8.5
N-CONs	75.2 ± 34.8	5.5 ± 7.3
A-CONs	58.9 ± 20.1	1.3 ± 0.8

Table S2. CHN elemental analysis for DhaTph, N-CONs and A-CONs.

	C	H	N
Theoretical (%)	77.07	4.10	11.98
DhaTph	74.77	4.34	11.52
N-CONs	75.28	4.23	11.83
A-CONs	74.78	4.22	11.79

Table S3. Apparent rate constants (k_{obs}) and quantum yields (AQY) for DPBF and Cyt c reactions.

	k_{obs} (DPBF, s^{-1})	AQY (DPBF, %)	AQY (Cyt c, %)
DhaTph	2.3×10^{-3}	5.8×10^{-2}	3.9×10^{-4}
N-CONs	2.7×10^{-3}	6.5×10^{-2}	4.9×10^{-4}
A-CONs	5.2×10^{-3}	1.1×10^{-1}	1.5×10^{-3}

Table S4. $^1O_2$ generation using various porphyrin-based materials.

Materials and specific topic	$^1O_2$ functions	Refs.
Porphyrin MOFs thin film	$^1O_2$ generated on the surface of film worked for antimicrobial activity.	S1
Porphyrin-photosensitizers in MOF cages	Antimicrobial activity for sustainable plant disease management.	S2
Porphyrin MOFs	Direct quantification. $^1O_2$ generation was estimated to be (0.35 ± 0.02).	S3
3D and 2D porphyrin COFs	3D COFs exhibited higher antimicrobial activity.	S4
Exfoliated porphyrin COFs	Exfoliated COFs exhibited enhanced antimicrobial activity, due to larger surface area.	This study

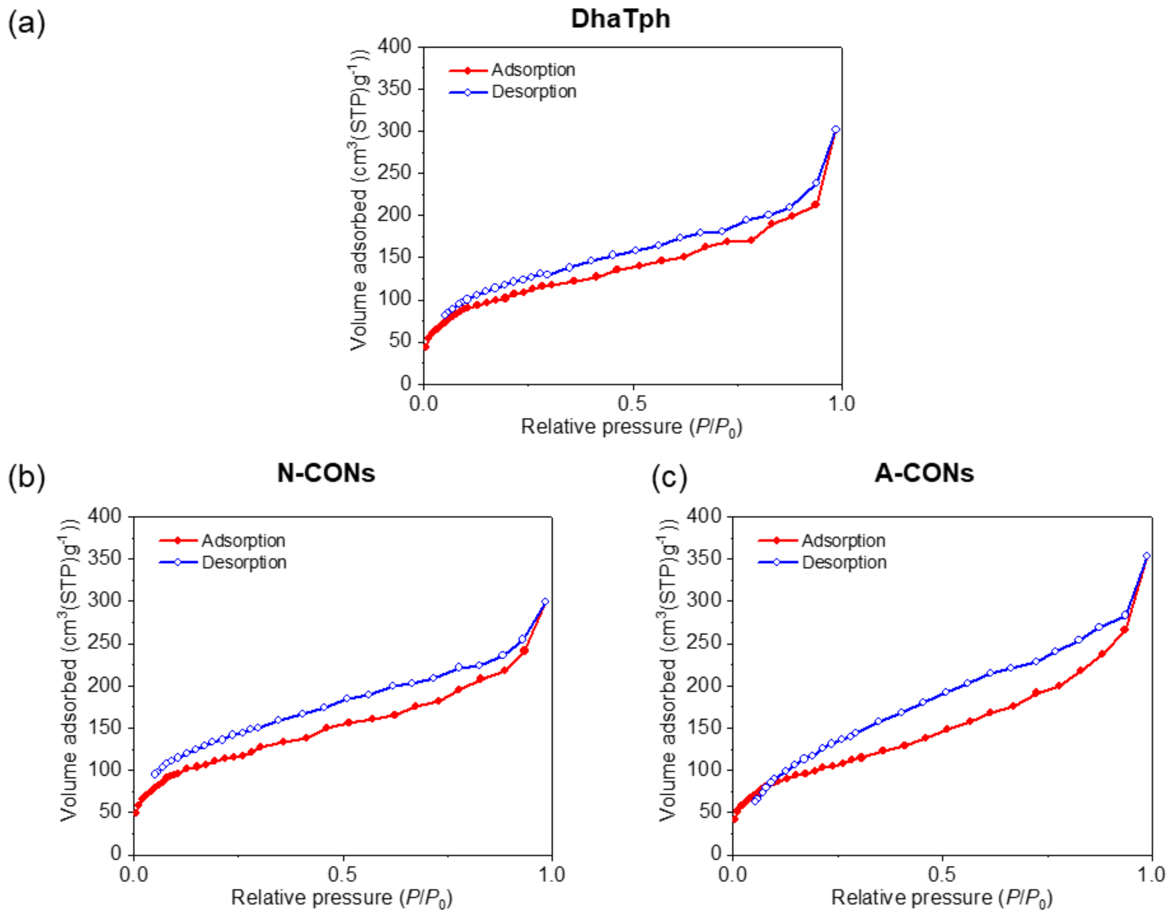

Fig. S1 BET nitrogen adsorption isotherm plots for DhaTph (a), N-CONs (b) and A-CONs (c).

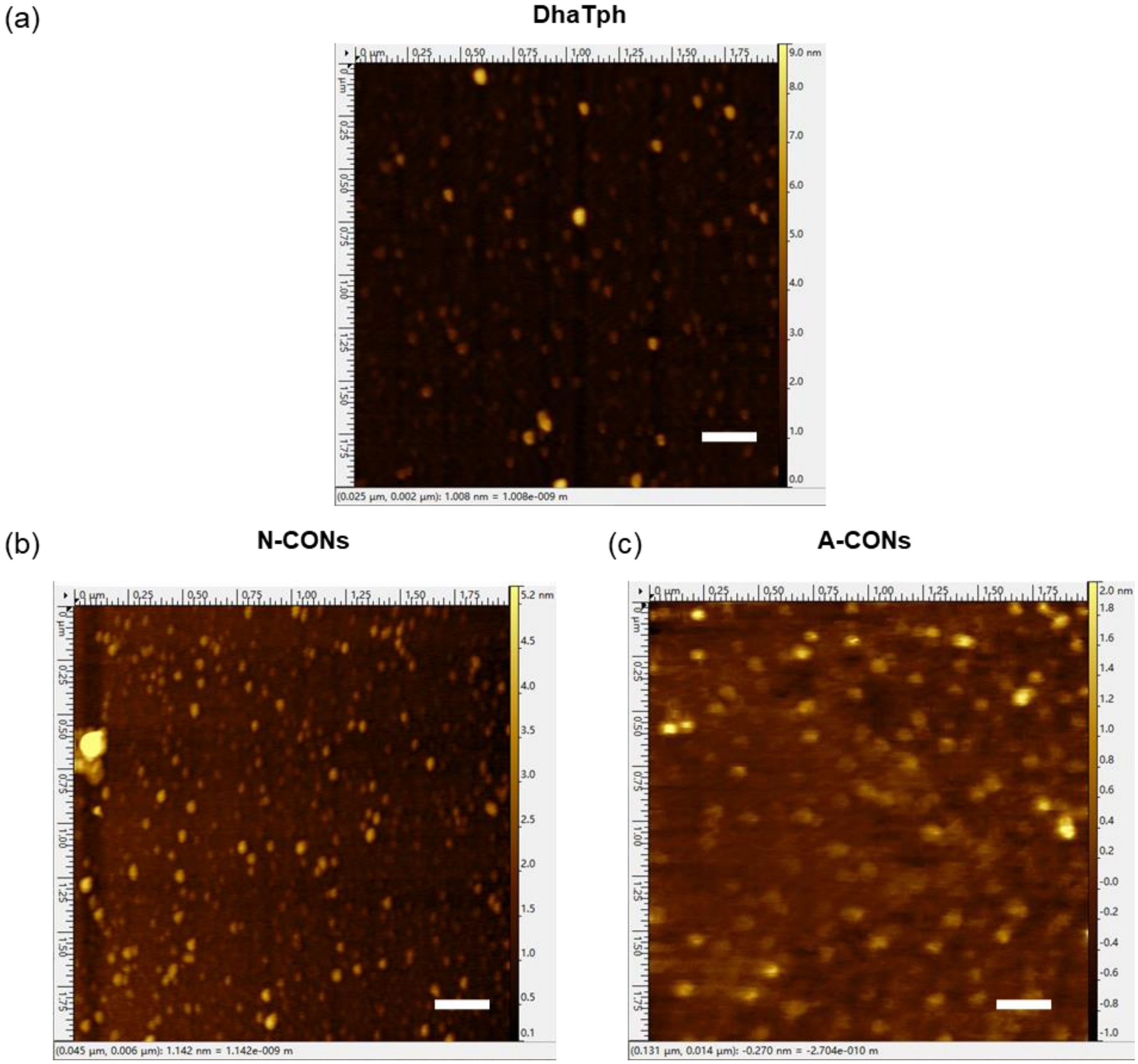

Fig. S2 Representative AFM images for DhaTph (a), N-CONs (b) and A-CONs (c). The scale bar is 250 nm.

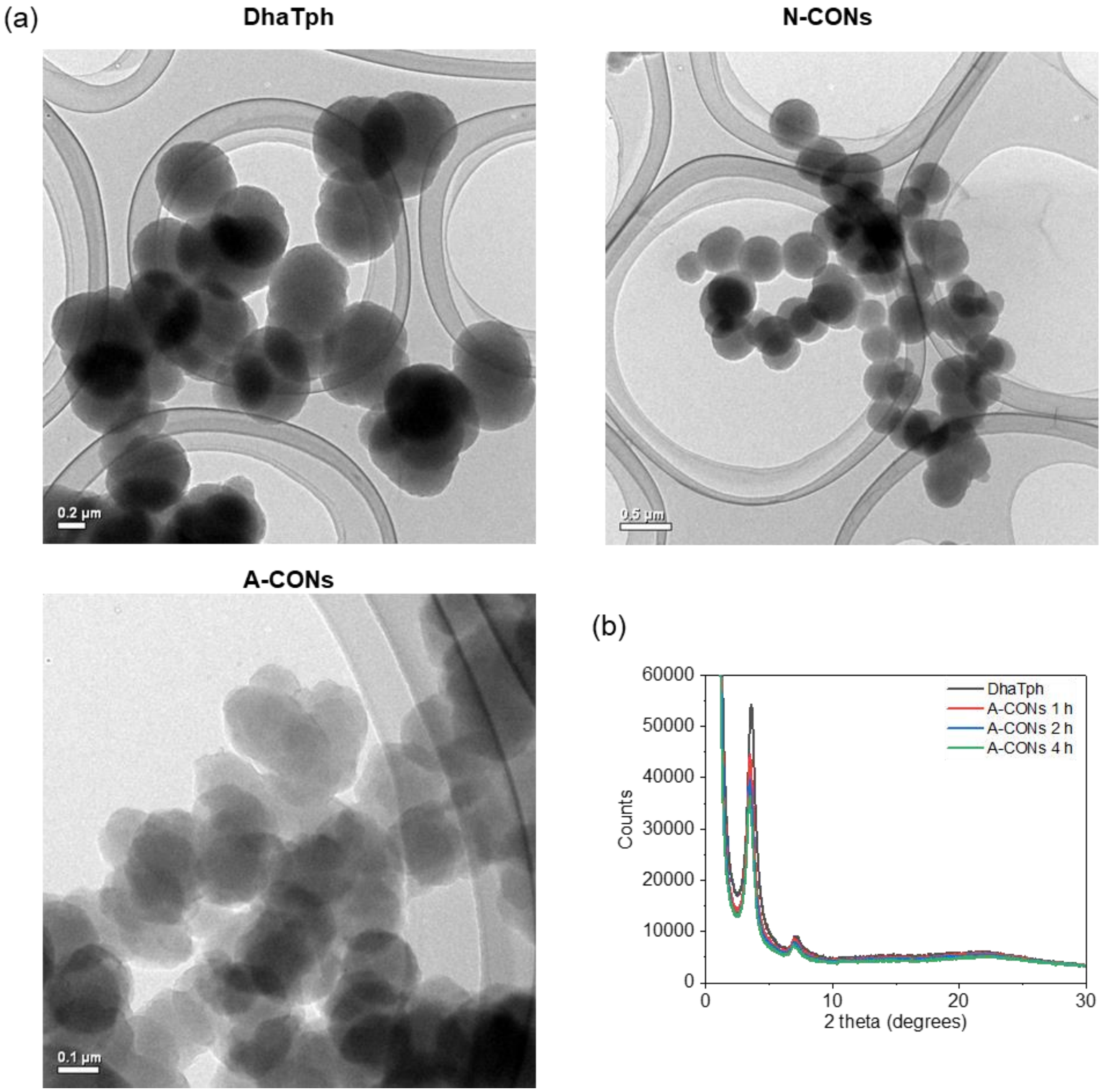

Fig. S3 (a) TEM images for DhaTph, N-CONs and A-CONs. (b) PXRD patterns for A-CONs reacted for 0, 1, 2, and 4 h.

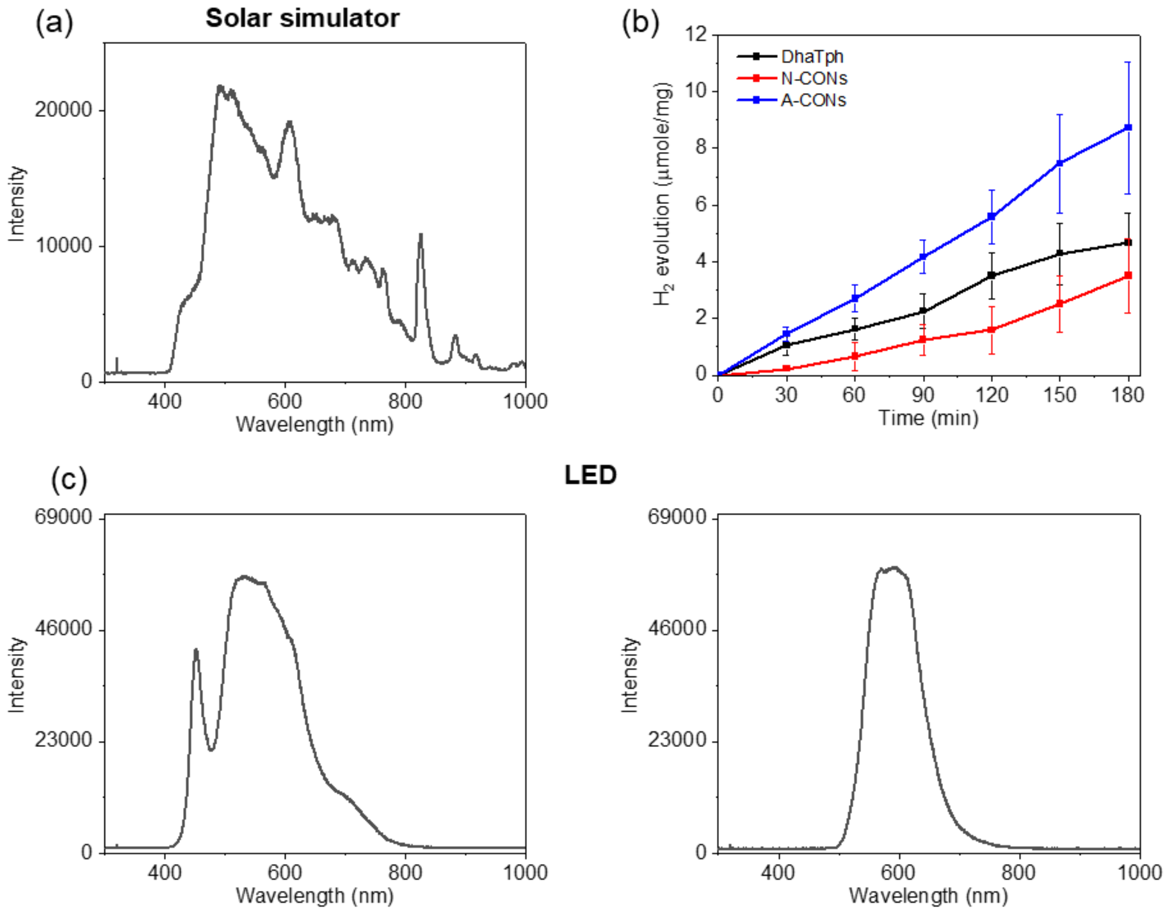

Fig. S4 (a) Emission spectra of light from solar light simulator. (b) H_2 yield for photocatalytic reaction in the presence of DhaTph/RGO/Pt, N-CONs/RGO/Pt, A-CONs/RGO/Pt. (c) Emission spectra for LED without yellow filter (left) and with yellow filter (right).

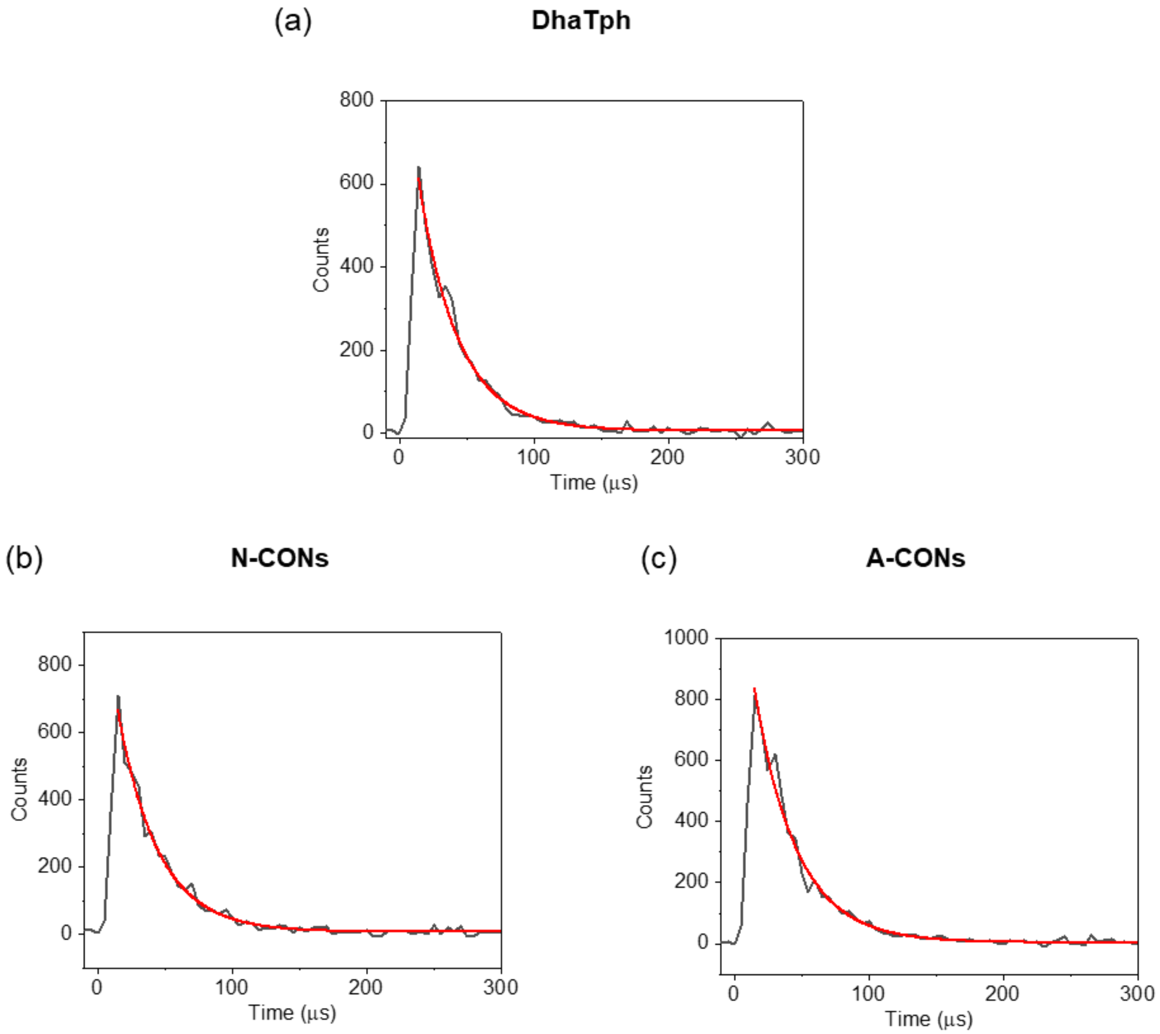

Fig. S5 Decay curves for emission at 1270 nm for DhaTph (a), N-CONs (b) and A-CONs (c).

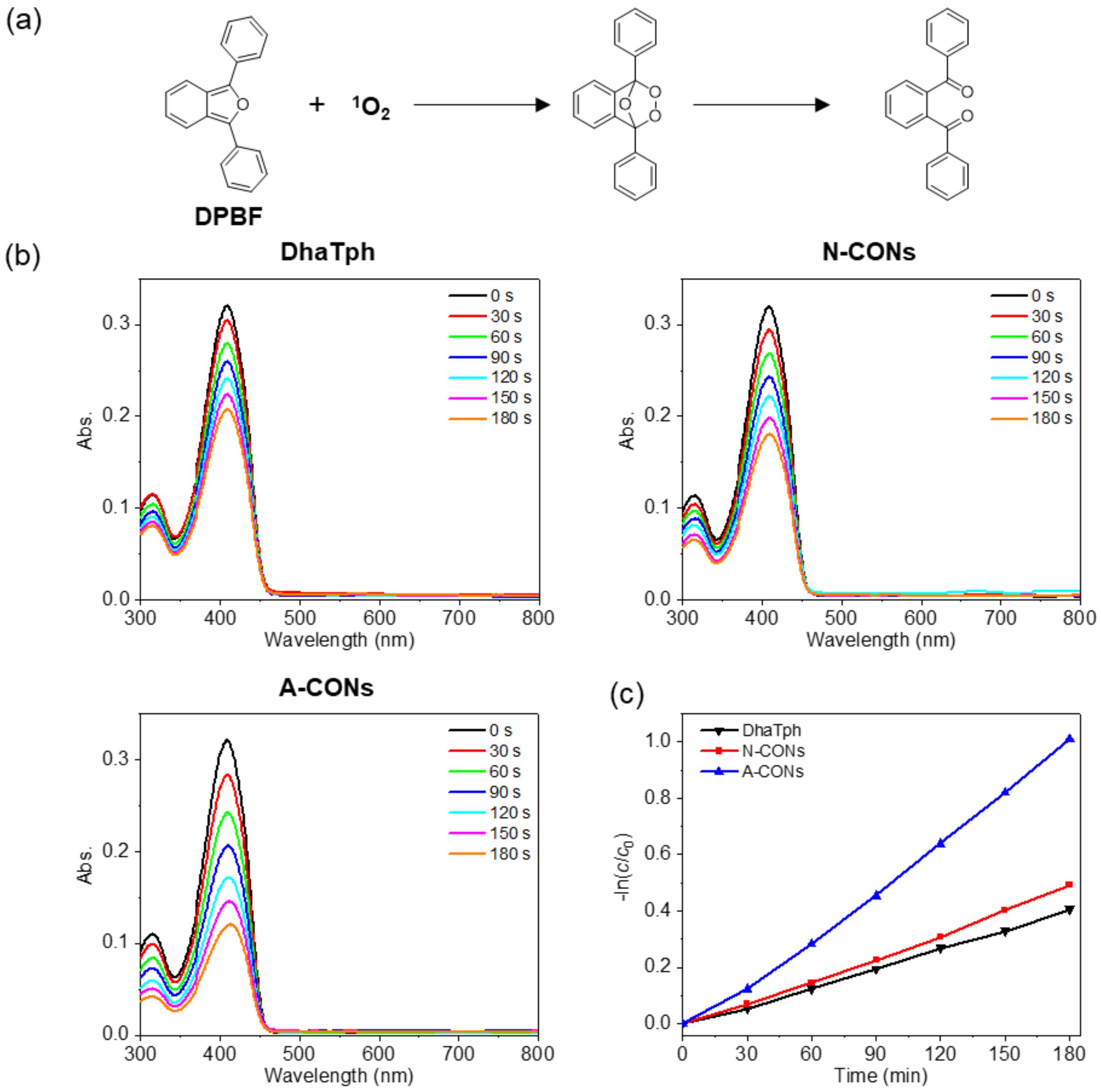

Fig. S6 (a) Reaction scheme of DPBF oxidation by $^1O_2$. (b) Absorption changes during BPBF reaction in the presence of DhaTph, N-CONs and A-CONs. (c) The plot of $-\ln(c/c_0)$ vs. time.

(a)

$$\text{Cyt c (Fe}^{3+}) + O_2^{\bullet -} \longrightarrow \text{Cyt c (Fe}^{2+}) + O_2$$

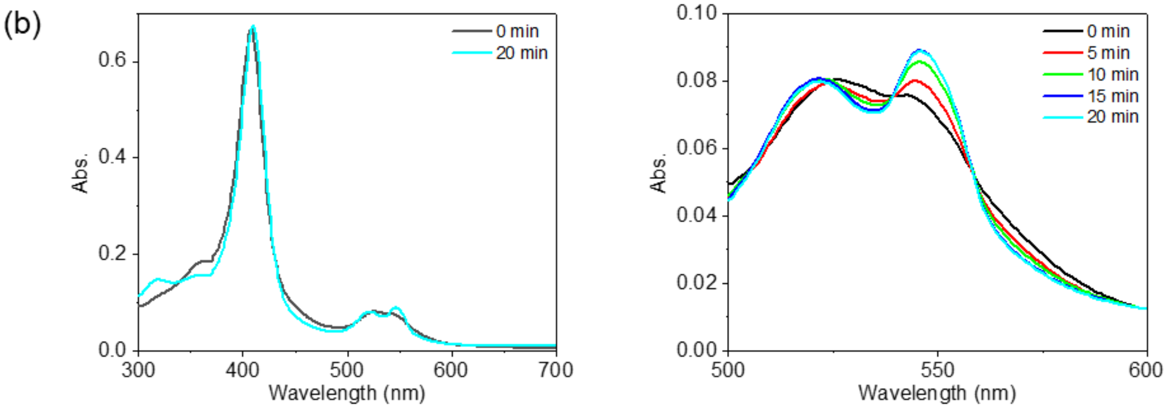

Fig. S7 (a) Cyt c reaction and (b) absorption changes during after photoirradiation in the presence of A-CONs.

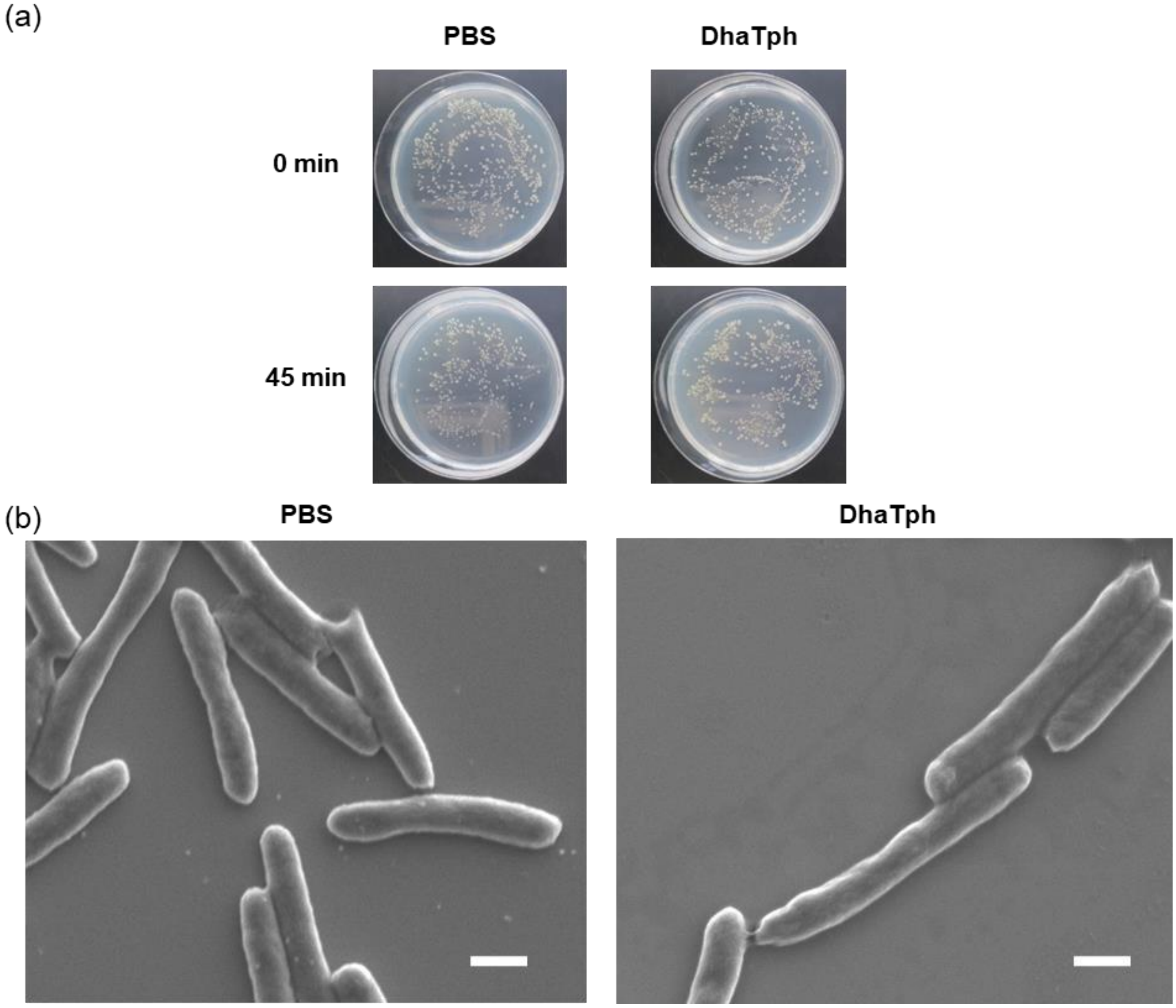

Fig. S8 (a) Representative plates of *E. coli* before and after photoirradiation with LED light in the absence or presence of DhaTph. (b) Represnetative SEM images of *E. coli* after photoirradiation in the absence or presence of DhaTph. Scale bar is 1 μm.

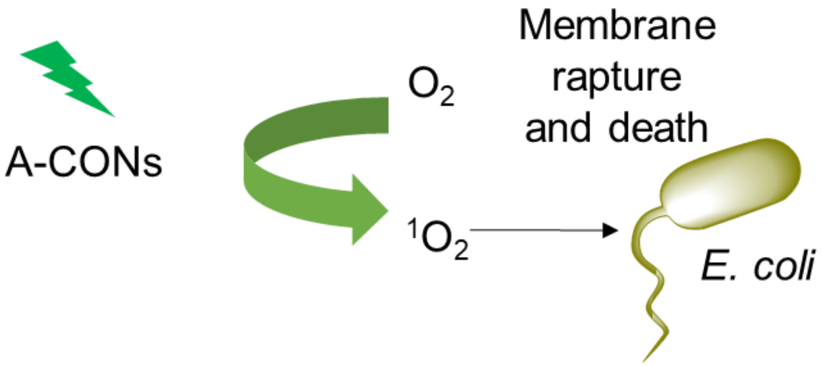

Fig. S9 Schematic illustration of bactericidal efficacy using A-CONs.

1. Materials and methods.

5,10,15,20−Tetrakis(4−aminophenyl)−21H, 23Hporphyrin (Tph) and 1,2-dichlorobenzene (DCB) were purchased from TCI (Tokyo, Japan). Acetic acid (HAc) was purchased from Nacalai Tesque (Kyoto, Japan). HAc/NaAc (pH=4.0) buffer was prepared by mixing HAc and $CH_3COONa/3H_2O$. 2,5-dimethoxy-1,4-dibenzaldehyde (Dma), and Cytochrome c (Cyt c) from bovine heart were purchased from Sigma-Aldrich (Missouri, USA). Other reagents were purchased from FUJIFILM Wako (Tokyo, Japan). All chemicals were used without further purification.

Powder X-ray diffraction (PXRD)

PXRD was measured on a SmartLab diffractometer (RIGAKU, Tokyo, Japan). The sample was placed on a glass sample holder with recessed well and the data was collected at a scan rate of 2°/min with Cu Kα radiation (λ = 1.5406 Å).

Fourier transform infrared (FT-IR) spectroscopy

FT-IR spectra were measured on a FT-IR 4100 spectrometer (JASCO, Tokyo, Japan). The recoding was conducted by 16 scans with a resolution of 4.0 cm^{-1} in attenuated total reflection mode.

UV-vis absorption measurements

Absorption spectra for solid sample was measured on V-770 UV-vis spectrometer (JASCO, Tokyo, Japan). Another measurement in solution was conducted using a V-630 Bio UV−vis spectrophotometer (JASCO, Tokyo, Japan).

Atomic force microscopy (AFM)

AFM images were measured by a SPI3800 instrument (Seiko, Chiba, Japan). Sample was dispersed in ***N*,*N***−dimethlformamide and sonicated for 10 min. The solution was dropped on freshly prepared mica plate (1 cm^2). The images were analyzed by Gwyddion free software (http://gwyddion.net/).

N_2 adsorption−desorption isotherm

N_2 adsorption –desorption isotherm was measured on a Quantachrome NOVA 4200e instrument (Quantachrome Instruments Japan G.K., Kanagawa, Japan).

Electron spin resonance (ESR) spectroscopy

ESR spectra were measured on an EMXmicro spectrometer (BRUKER, MA, USA). The spin probes were dissolved in H_2O at the concentration of 50 μM as stock solution. The 1 mL of stock solution was added to 1 mg A-CONs and sonicated for 5 min before photoirradiation. The sample was photoirradiated and centrifuged to remove the A-CONs. The supernatant of 100 μL was transferred to ESR tube and the spectra was measured as previously described.[5]

2. Experimental procedures.

Synthesis of DhaTph.

DhaTph was synthesized as previously described.[6] In brief, dihydroxyterephthalaldehyde (Dha, 28 mg) synthesized from Dma, and Tph (56 mg) was reacted in the 30 mL mixture solution of DCB: ethanol: HAc (6 N) = 5:5:1 at the boiling point for three days. After cooling down to room temperature, the reactant was filtrated and the resulting solid was washed by 5 mL of ethanol × 5 and dried under vacuum at 120°C.

Synthesis of N-CONs.

DhaTph (20 mg) was reacted in 20 mL water under stirring for 2 hours at room temperature. The reactant was filtered and washed with 5 mL of ethanol × 5.

Synthesis of A-CONs.

DhaTph (20 mg) was reacted in 20 mL of 0.2 M HAc/NaAc (pH=4.0) buffer under stirring for 2 hours at room temperature. The reactant was filtered and washed with 5 mL of ethanol × 5.

Hydrogen evolution reaction (HER)

Hydrogen evolution was quantified as previously described.[7] In brief, 0.5 mL sample (0.5 mg/mL in methanol), 0.25 mL of Pt/reduced graphene oxide and 4.25 mL water were added into a 30 mL test tube. After sonication for 10 min and Ar bubbling for 30 min, the test tube was photoirradiated with a HAL-320W solar simulator (Asahi-bunko, Tokyo, Japan) equipped with 420 nm long-pass filter. The gas sampling was conducted every 30 min and analyzed by a GC-8A gas chromatograph (Shimadzu, Kyoto, Japan) equipped with an MS-5A column.

Direct measurement of $^1O_2$

The sample was prepared at the concentration of 0.2 mg/mL in toluene and sonicated for 10 min. After transferring into 1×1 cm^2 quartz cell, the sample was bubbled by O_2 or Ar gas for 20 min before the measurements. $^1O_2$ was measured by the direct observation of phosphorescence signal from $^1O_2$ using single-photon counting as previously described.[8] We measured the phosphorescence upon 532 nm nanosecond laser excitation from a Q-switched Nd:YAG laser (Continuum, Surelite II-10, fwhm 4 ns). The phosphorescence spectra was measured at a delay of 70 μs with a gate width of 5 μs. The kinetic trace was measured with a gate width of 10 μs at 1270 nm.

DPBF oxidation for $^1O_2$ assay

1 mg sample was introduced into 4 mL of 50 μM DPBF in methanol. The suspension was photoirradiated by a LED light (W-LITE, amazon Japan) with yellow filter ($\lambda > 550$ nm) at room temperature and measured the absorption spectra. We note that we did not conducted the O_2 bubbling for $^1O_2$ assay.

Cyt c reduction for $O_2^{\bullet -}$ assay

1mg of A-CONs was added to 4 mL of 20 μM Cyt c aqueous solution in a test tube and the test tube was subjected to O_2-bubbling before light irradiation. The suspension was photoirradiated by the LED light with yellow filter (λ > 550 nm). The 1 mL of suspension was centrifuged at 10, 000 rpm on mini spin (Eppendorf Japan, Tokyo, Japan) for 5 min, and the supernatant was analyzed by UV-vis spectrometer.

***Escherichia coli* (*E. coli*) survival rate quantification**

E. coli DH5α competent cells (Takara Bio, Shiga, Japan) was harvested in 5 mL LB medium at 37°C in a BR-23FP bioshaker (108 rpm/min, TAITEC, Osaka, Japan). Sample (0.5 mg/0.5 mL in D-PBS) was added to *E. coli* suspension at the density of $1{,}500 \times 10^3$ CFU/mL in 5 mL of D-PBS. The suspension was photoirradiated by LED light with yellow filter (λ > 550 nm) and 100 μL was added to 1 mL LB medium, subjected to the plating of 20 μL on Agar plate of 10 cm and overnight incubation at 37°C. The photograph of individual Agar plate was taken and the number of colonies were counted by Image J software.

***E. coli* morphology characterization under a scanning electron microscopy (SEM)**

E. coli suspension photoirradiated by the above method was fixed and plated on silicon chip for SEM observation. In brief, the *E. coli* was collected by centrifugation at the speed of 4,000 g for 10 min (himac high-speed micro centrifuge, CF15NR, Ibaraki, Japan). After the centrifugation, the supernatant was removed and the 2.5% glutaraldehyde in D-PBS was added to the tube for the fixation for two hours at room temperature. After the centrifugation of the tube, the *E. coli* was collected and washed again, and dehydrated with ethanol for 10 min each using different concentration at 30, 50, 70, 80, 90 and 100%. After the centrifugation, the pellet remained in ethanol was dropped on silicon chip for 5 μL × 2. After completely dried out the silicon chip, the chip was coated with osmium and observed by JSM-F100 (JEOL, Tokyo, Japan).

Supporting references.

S1. W. Zhou, S. Begum, Z. Wang, P. Krolla, D. Wagner, S. Bräse, C. Wöll and M. Tsotsalas, *ACS Appl. Mater. Interfaces*, 2018, **10**, 1528-1533.

S2. J. Tang, G. Tang, J. Niu, J. Yang, Z. Zhou, Y. Gao, X. Chen, Y. Tian, Y. Li, J. Li and Y. Cao, *J. Agric. Food. Chem.*, 2021, **69**, 2382-2391.

S3. Y. Hao, B. M. Liu, T. F. Bennett, C. G. Monsour, M. Selke and Y. Liu, *J. Phys. Chem. C*, 2021, **125**, 7392-7400.

S4. J. Hynek, J. Zelenka, J. Rathouský, P. Kubát, T. Ruml, J. Demel and K. Lang, *ACS Appl. Mater. Interfaces*, 2018, **10**, 8527-8535.

S5. H. Shigemitsu, T. Tamemoto, K. Ohkubo, T. Mori, Y. Osakada, M. Fujitsuka and T. Kida, *Chem. Commun.*, 2021, **57**, 11217-11220.

S6. S. Kandambeth, D. B. Shinde, M. K. Panda, B. Lukose, T. Heine and R. Banerjee, *Angew. Chem., Int. Ed.*, 2013, **52**, 13052-13056.

S7. X. Li, T. Goto, K. Nomura, M. Zhu, T. Sekino and Y. Osakada, *Appl. Surf. Sci.*, 2020, **513**, 145720.

S8. Y. Osakada, K. Kawai, T. Tachikawa, M. Fujitsuka, K. Tainaka, S. Tero-Kubota and T. Majima, *Chem. Eur. J*, 2012, **18**, 1060-1063.