Supporting Information

Photoswitchable Phthalocyanine-Assembled Nanoparticles for Controlled “Double-Lock” Antibacterial Photodynamic Activity

Hong-Bo Cheng,+[a] Xingshu Li,[a] Nahyun Kwon,[a] Yanyan Fang,*[b] Gain Baek[a] and Juyoung Yoon*[a]

[a] Dr. H. B Cheng, Dr. X. Li, N. Kwon, G. Baek, Prof. J. Yoon
Department of Chemistry and Nano Science
Ewha Womans University, Seoul120-750, Korea
E-mail: jyoon@ewha.ac.kr

[b] Dr. Y. Fang
Beijing National laboratory for Molecular Sciences, Key Laboratory of Photochemistry, Institute of Chemistry
Chinese Academy of Sciences
Beijing 100190 (China)
E-mail: fyy001@iccas.ac.cn
Materials and methods

All chemicals were commercially available unless noted otherwise. All reagents were used as received. Representative phthalocyanine ZnPcs was prepared according to our previously described procedures [1]. UV absorption spectra were obtained on a VIKON 933 Double Beam UV/VIS Spectrometer. Fluorescence emission spectra were obtained using a RF-5301/PC Spectro fluorophotometer (Shimadzu). $^1$H NMR spectra were determined using a Bruker AVANCE III 400 spectrometer (400 MHz). High-resolution mass spectra (HRMS) were determined using an Exactive Plus Orbitrap (Thermo Fisher Scientific). Dynamic light scattering (DLS) was measured using a Nano-ZS (Malvern). TEM images were recorded on a JEOL-2100F electron microscope operating at 200 kV.

ROS detection: Detection of ROS was performed using 2,7-dichlorofluorescin diacetate as the probe. In the presence of ROS (e.g. $^{1}\text{O}_2$), 2,7-dichlorofluorescin diacetate is oxidized to form dichlorofluorescein which emits bright fluorescence around 520 nm. Briefly, photosensitizer (5 μM) and 2,7-dichlorofluorescin diacetate (24 μM) were dissolved in water. A water solution only containing 2,7-dichlorofluorescin diacetate was used as the control. The mixtures were irradiated by using a 500 W halogen lamp and a water tank for cooling. After various irradiation times, fluorescence spectra (excited at 504 nm) of the mixtures were recorded using a RF-5301/PC Spectrofluorophotometer.

Photodynamic antibacterial effects: S. aureus (ATCC 25923), E. coli (ATCC BAA-198) were used for antibacterial determinations. Pathogens were grown overnight in a round glass tube containing Mueller-Hinton Broth (not cation-adjusted) for Gram-positive bacteria or Luria-Bertani Broth for Gram-negative bacteria at 37 °C and 42 rcf. The cultures were then diluted 100 times with fresh media and continuously grown at 37 °C until optical densities (OD) at 600 nm reached 1. Each culture solution was centrifuged at 5000 rpm for 10 min. The supernatant was discarded and precipitate was washed with PBS two times and re-suspended in 1 mL PBS to prepare a stock solution. For antibacterial determinations, cells (50 μL of stock solution) were
mixed with NanoPcA or MB at various concentrations (0-100 nM) in 500 μL of total solution and incubated at 37 °C for 2 h. Then, 200 μL of each mixture was irradiated with laser (655 nm, 0.4 W/cm2) for 10 min, and the resultant bacterial suspension was diluted 10 times with PBS and plated on a LB agar plate to determine the CFUs/mL. Numbers of viable bacteria were then determined by using the conventional plate counting method.

Cryo TEM detection: E. coli were cultured overnight in LB medium at 37 °C and 42 rcf. The culture was then diluted 100 times with fresh media and continuously grown at 37 °C until its optical density (OD) at 600 nm reached 1. A 1 mL aliquot of the resulting bacterial culture solution centrifuged at 5000 rpm for 5 min. The supernatant was discarded and the precipitate was washed with PBS two times and resuspended in 1 mL PBS to prepare a stock solution. E. coli bacteria were incubated with 365 nm irradiated NanoAzoPcS at 37 °C for 2 h and 200 μL of the mixture was irradiated with laser (655 nm, 0.4 W/cm2) for 10 min. The resultant bacteria suspension was centrifuged and the supernatant was discarded. The precipitate was further washed with PBS two times to remove the unbound NanoAzoPcS and re-suspended in PBS. Bacteria were collected using 0.25% trypsin-1 mM EDTA and washed three times with PBS, centrifuged into a small pellet and fixed in 2.5 % glutaraldehyde in phosphate buffer, pH 7.2. The collected cells were fixed from 4 h to overnight at 4 °C in a refrigerator. Post fixation, cells were suspended in 2% aqueous OsO4/0.1M PBS (pH 7.4), washed 3 times for 5 minutes in 0.1M PBS, and dehydrated in graded series of ETOH - 50%, 70%, 95%, 100% ETOH (2 times) for 15 min and propylene oxide (2 times) for 15 min. The samples were treated overnight with propylene oxide/Epon resin, and infiltrated with fresh 100% Epon resin for 1-2 h and then embedded in beem capsules. Samples were polymerized in Beem capsules at 60 °C for 12-24 h. Thick sections (1 micron) were scanned to find the area of interest and ultra thin sections (~70nm) were then obtained (LEICHER ULTRACUT S;Leica company.) and placed on copper grids. The samples in the copper grids were electro-stained with uranyl acetate for 5 min, and lead citrate for 2 min. The samples were then observed by using a Cryo TEM (FEI; CryoTecnai F20) in Korea Institute of Science and
Technology.

**Synthesis and characterization of Guest 1**

![Synthesis pathway to prepare Guest 1.](image)

**Figure S1** Synthesis pathway to prepare Guest 1.

Under Ar atmosphere, compound 4 (0.50 g, 1.17 mmol) was added to triethylamine (50 ml 33% ethanol solution). The reaction mixture was stirred for 12 hr at 80 °C. Then, the reaction mixture was cooled down and the excess triethylamine and ethanol were removed under reduced pressure. The collected yellow solid was recrystallized with methanol/diethyl ether to give the target compound 1 as a yellow solid (0.15 g, yield 75%). $^1$H NMR (300 MHz, DMSO) $\delta$ 7.85 (d, $J = 9.3$ Hz, 4H), 7.14 (d, $J = 9.1$ Hz, 4H), 4.15 (t, $J = 5.4$ Hz, 4H), 2.51 (s, 16H), 1.82 (s, 8H), 1.19 (t, $J = 7.2$ Hz, 18H). $^{13}$C NMR (75 MHz, DMSO) $\delta$ 161.77, 141.40, 127.22, 124.13, 115.32, 104.63, 67.66, 61.31, 52.63, 28.10, 15.57, 7.44. HRMS (ESI) $m/z$ [M – 2Br]$^{2+}$ calcd for C$_{32}$H$_{54}$N$_4$O$_2$$^{2+}$ 263.2118, found 263.21596.

**Figure S2** The HRMS spectrum of Guest 1.
Synthesis and characterization of Guest 2

![Chemical structure](image1.png)

**Figure S3** Synthesis pathway to prepare Guest 2.

4, 4’-azopyridine (0.500 g, 2.65 mmol) and benzyl bromide (0.70 ml, 5.8 mmol) was dissolved in acetonitrile (50 mL) and refluxed at 80 °C for 24 h. Then cooled to 0 °C and brown precipitate was separated by filtration, yielded guest 4 (yield 63%). \( ^1H \) NMR (300 MHz, DMSO) \( \delta 9.16 \) (d, \( J = 5.5 \) Hz, 4H), 8.63 (t, \( J = 7.8 \) Hz, 2H), 8.31 – 8.06 (m, 4H), 7.84 (d, \( J = 9.0 \) Hz, 4H), 7.11 (d, \( J = 9.1 \) Hz, 4H), 4.73 (t, \( J = 7.3 \) Hz, 4H), 4.13 (t, \( J = 6.2 \) Hz, 4H), 2.25 – 2.00 (m, 4H), 1.89 – 1.57 (m, 4H). HRMS (ESI) \( m/z [M – 2Br]^2+ \) calcd for C\(_{24}\)H\(_{22}\)N\(_4^2+\) 183.0917, found 183.09231.

Synthesis and characterization of Guest 3

![Chemical structure](image2.png)

**Figure S4** Synthesis pathway to prepare Guest 3.

Transfer excess 2-chloroethanol (51 mL, 0.76 mol) and pyridine (53 mL, 0.63 mol) to a 250 mL round bottom flask which is fitted with reflux condenser and nitrogen protecting facilities, and the reaction mixture was gently stirred at 70 °C for 24 h in the dark, the crude product was formed. Then the crude product was purified by the recrystallization with the solvent of acetonitrile and ethyl acetate, and the residual solvent was removed in vacuum to give the product as a white crystal (yield 55%). \( ^1H \) NMR (300 MHz, DMSO) \( \delta 9.16 \) (d, \( J = 5.5 \) Hz, 4H), 8.63 (t, \( J = 7.8 \) Hz, 2H), 8.31 – 8.06 (m, 4H), 7.84 (d, \( J = 9.0 \) Hz, 4H), 7.11 (d, \( J = 9.1 \) Hz, 4H), 4.73 (t, \( J = 7.3 \) Hz, 4H), 4.13 (t, \( J = 6.2 \) Hz, 4H), 2.25 – 2.00 (m, 4H), 1.89 – 1.57 (m, 4H). HRMS (ESI)
m/z [M – Br]$^+$ calcd for $\text{C}_9\text{H}_{14}\text{N}^+$ 136.1121, found 136.11713.

**Figure S5** Change in the absorption spectrum of host ZnPcS (5 µM) in water upon titration with Guest 2 from 0-35 µM.
Figure S6 Change in the absorption spectrum of host ZnPcS (5 µM) in water upon titration with Guest 3 from 0-35 µM.

Figure S7 Change in fluorescence spectra of host ZnPcS (5 µM) in water upon titration with Guest 2 from 0-35 µM.
Figure S8 Change in fluorescence spectra of host ZnPcS (5 µM) in water upon titration with Guest 3 from 0-35 µM.

Figure S9 Possible structure of the assembly of azo 1 after sufficient photoirradiation at 365 nm and 450 nm.
**Figure S10** Absorption of azo 1 after photoirradiation at 365 nm.

**Figure S11** Intensity changes of azo 1 (5 µM) upon alternating UV (365 nm) and visible light (450 nm) irradiation in water.
**Figure S12** ROS generation by irradiation of locking NanoAzoPcS (ZnPcS/azo 1 = 5 µM/35 µM) in water solutions detected using 2,7-dichlorofluorescin diacetate as the fluorescent probe.

**Figure S13** ROS generation by irradiation of unlocking NanoAzoPcS (ZnPcS/azo 1 = 5 µM/35 µM) in water solutions detected using 2,7-dichlorofluorescin diacetate as the fluorescent probe.
**Figure S14.** Cryo TEM images of *S. aureus* bacterial cells treated with unlocking NanoAzoPcS (ZnPcs/azo 1 = 5 µM/35 µM). Red arrows indicate the small nanoparticles after dissociation of NanoAzoPcS.

**Plate Photographs of *E. coli* on LB agar plate**

<table>
<thead>
<tr>
<th>Before laser irradiation</th>
<th>After laser irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Guest</strong></td>
<td><strong>NanoAzoPcs</strong></td>
</tr>
<tr>
<td><strong>Unlocking NanoAzoPcs</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Figure S15** Plate photographs of *E. coli* on LB agar plate treated with Guest 1, locking NanoAzoPcS (ZnPcS/azo 1 = 5 µM/35 µM), unlocking NanoAzoPcS (ZnPcs/azo 1 = 5 µM/35 µM) under laser irradiation. Control groups are bacteria without any treatment. Laser conditions: 655 nm, 0.4 W/cm², 10 min.
Figure S16. Controlled photodynamic antibacterial effects. *S. aureus* photoinactivation in the presence of different concentrations of unlocking NanoAzoPcS (ZnPcS/azo 1 = 5 µM/35 µM) after laser irradiation (655 nm, 0.4 W/cm², 10 min).

Notes and references