Electronic Supplementary Information

Photodynamic Inactivation of *Escherichia coli* by Porphyrin Cytochrome c

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**Experimental section**

Porphyrin Cyt c was prepared using a literature method. 10 mL of HF-pyridine (70% HF) for every 30 mg of lyophilized horse heart Cyt c were placed in a Teflon beaver, stirred for 10 min at room temperature. Then nitrogen was flowed over the beaver to remove the HF. When all HF was removed, a fluorescent, purple-colored paste was left. This was dissolved in a small amount of 10 mM sodium acetate (pH = 5.0) and dialyzed against distilled water for 24 h. Porphyrin Cyt c was obtained by lyophilized the dialysate. All preparations were done in minimal light.

UV-Vis absorption spectra were recorded on a Shimadzu UV-1601 spectrophotometer. Fluorescence emission spectra were run on a Hitachi F-4500 fluorescence spectrophotometer. The singlet oxygen quantum yields of the proteins in PBS were determined by using TPPS (0.62 in PBS) as the reference and AMDA as the trapping agent of singlet oxygen. Transmission electron microscopy (TEM) images were taken by JEOL JEM-2100 LaB6 microscope operated at an accelerating voltage of 200 kV. Fluorescent micrograms were performed on a Nikon C1Si inverted fluorescent microscope and the magnification employed was 10 × 60. The Zeta potentials were measured in PBS suspensions on a Malvern Zetasizer 3000HS (Malvern Instruments Ltd).

A single colony of *E. coli* (JM109) on a solid Luria-Bertani (LB) agar plate was transferred to 8 mL of liquid LB culture medium and was grown at 37 °C for 16 h. Bacteria were harvested by centrifuging at 8000 rpm for 10 min and washing twice with 5 mM PBS at pH 7.0. The supernatant was discarded and the *E. coli* pellets were resuspended in PBS at a density of 10⁶-10⁸
cells/mL. The phototoxicity of the porphyrin Cyt c were determined by incubation with *E. coli* cell suspensions for 10 min in the dark at 37 °C and then exposed to an irradiation of ≥ 400 nm for 1 h (using an Oriel 91192 Solar stimulator as the light source and a 400 nm cut-off filter to remove the short wavelength light. The irradiation intensity was about 14 mW/cm² and the total light dose was approximately 50 J/cm²). The treated bacterial samples were diluted 10⁶-fold in PBS and were spread on 3M Petrifilm™ *E. coli* Count Plate and incubated at 37 °C for 48 h. The number of colony-forming units (CFU) was counted by a Shineso G6 Colony Counter.

**Binding of porphyrin Cyt c onto *E. coli* cells.**

![Figure S1](image1.png)

**Figure S1.** TEM micrographs of *E. coli* before (a) and after (b) the addition of 10 μM porphyrin Cyt c.

![Figure S2](image2.png)

**Figure S2.** Bright-field microgram (a) and fluorescence microgram (b) of *E. coli* incubated with 10 μM porphyrin Cyt c for 10 min in the dark (λex = 405 nm). (c) Overlay of (a) and (b).
Photoinactivation of *E. coli*.

**Figure S3.** Dark toxicity of porphyrin Cyt c (0-10 μM) upon *E. coli*.

**Figure S4.** Colony forming units (CFU) of *E. coli* on 3M Petrifilm™ *E. coli* Count Plate. (a) *E. coli* suspensions only with 1 h of light irradiation. (b) *E. coli* suspensions were incubated with 10 μM Cyt c for 10 min and then exposed to visible light for 1 h. (c) *E. coli* suspensions were incubated with 10 μM PPIX for 10 min and then exposed to visible light for 1 h.
Structure similarity of the porphyrin in Cyt c and PPIX.

Cytochrome c

PPIX

Figure S5. Porphyrin and amino acid sequence of Cyt c and the structure of PPIX.