Electronic Supplementary Information

Light-up probe with aggregation-induced emission characteristics for selective imaging, naked-eye detection and photodynamic killing of Gram-positive bacteria

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Materials: Vancomycin, propargylamine, copper(II) sulfate ($CuSO_4$), sodium ascorbate, 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), *N*-hydroxysuccinimide (NHS), *N*, *N*-diisopropylethylamine (DIPEA), anhydrous dimethyl sulfate (DMSO), anhydrous dimethylformamide (DMF) and other chemicals were all purchased from Sigma-Aldrich and used as received without further purification. All non-aqueous reactions were carried out under nitrogen atmosphere in oven-dried glassware. Milli-Q water was supplied by Milli-Q Plus System (Millipore Corporation, Bedford, USA). Phosphate-buffer saline (PBS; 10 ×) buffer with pH 7.4 (ultrapure grade) is a commercial product of 1st BASE Singapore. *E. coli* (ATCC 25922), *B. subtilis* (ATCC 33677), Enterococcus faecium (Van A, genotype, ATCC 51559), and Enterococcus faecalis (Van B, ATCC 51299) were provided by American Type Culture Collection.

Characterization: UV-vis spectra were recorded on a Shimadzu UV-1700 spectrometer. Photoluminescence (PL) spectra were measured on a Perkin Elmer LS-55 equipped with a xenon lamp excitation source and a Hamamatsu (Japan) 928 PMT, using 90 degree angle detection for solution samples. All the UV-vis and PL spectra were collected at 24 ± 1 °C. The morphology of bacteria was observed by scanning electron microscope (SEM) (JSM-6700F, JEOL, Japan) at an accelerating voltage of 5 kV. Bacteria were fixed on a stub with a double-sided sticky tape and then coated with a platinum layer using an auto-fine coater (JEOL, Tokyo, Japan) for 60 s in a vacuum at a current intensity of 30 mA.

Synthesis of graphene oxide (GO): GO was synthesized by a modified Hummers method.[1] Briefly, into the mixture of 500 mg of graphite powder in an ice-bath, 700 mg of $NaNO_3$ and 3.1 g of $KMnO_4$ in35 mL of sulfuric acid was added dropwise.. The mixture was stirred at 35 ^oC for 1.5 hours, then 40 mL of water was added gradually. After further stirring at 95 ^oC for 1.5 hours, another 100 mL of water was slowly added, which was followed by the addition of 3.6 mL of H_2O_2 (36 %). The reaction color was turned from dark brown to yellow. After repeated washing and dialysis to remove acid, GO was obtained. The concentration of GO was determined by lyophilization of a certain volume of the GO solution. The concentration of GO used in this work is 1.7 mg/mL.

Synthesis of AIE-2Van. Alkyne-functionalized vancomycin was first prepared according to the following procedure: vancomycin (40 mg, 26.8 μmol), propargylamine (9.8 mg, 107.2 μmol) and DIPEA (1 μL) were dissolved in anhydrous DMSO/DMF (v/v = 1/1, 1.0 mL) and stirred at room temperature for 10 minutes. Then EDC (20.6 mg, 107.2 μmol) and NHS (6.2 mg, 107.2 μmol) were added to the above solution under nitrogen atmosphere. The reaction was performed under nitrogen atmosphere for 24 h at room temperature. After that, the reaction mixture was extensively dialyzed (SpectraPor 6, molecular weight cutoff of 1,000) against deionized water to remove EDC, NHS and propargylamine. The alkyne-functionalized vancomycin was obtained as white powders after freeze-drying under vacuum.

The AIE fluorogen was synthesized according to our previous report.[2] ^{1}H NMR (400 MHz, DMSO-d6): δ 7.63 (t, J = 7.5 Hz, 1H), 7.55 (t, J = 7.6 Hz, 2H), 7.42 (d, J = 7.3 Hz, 2H), 7.25 (d, J = 8.4 Hz, 2H), 7.19 (t, J = 7.1 Hz, 2H), 7.14 (d, J = 7.2 Hz, 1H), 7.09 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 7.0 Hz, 2H), 6.86 (m, 4H), 6.72 (m, 4H), 3.95 (m, 4H), 3.48 (m, 4H), 1.94 (m, 4H); 13C NMR (100 MHz, CDCl3): δ 174.40, 157.87, 157.61, 149.65, 143.18, 142.65, 137.86, 136.17, 135.75, 135.71, 133.42, 132.73, 132.65, 132.44, 131.65, 131.37, 130.43, 130.06, 128.75, 128.05, 126.65, 114.16, 114.07, 113.85, 113.63, 80.58, 64.42, 64.34, 48.24, 28.79.

Alkyne-functionalized vancomycin (20 mg, 12.2 μmol) and the AIE flurogen (2.2 mg, 3.1 μmol) were dissolved in a mixture of dimethyl sulfoxide and water (v/v = 5/1, 1.0 mL). The “click” reaction was initiated by sequential addition of $CuSO_4$ (1.9 mg, 12 μmol) and sodium ascorbate (4.8 mg, 24 μmol). The reaction was continued with stirring at room temperature for another 24 h. The final product was purified by HPLC and lyophilized under vacuum to yield the probe (1.7 mg, 24% yield) as a red powder. HPLC (λ = 320 nm): purity 96.4%, ESI-MS: m/z $[M+3H]^{3+}$ calc. 1218.067, found 1218.721.

Bacteria culture: A single colony of bacteria is picked up from the solid LB agar plate and transferred to liquid LB medium (5 mL). The bacteria suspension is cultured in an incubator at 37 °C. After 16 to 18 h to culture, the bacteria suspension is centrifuged (5000 rpm, 5 min) and washed three times with 1×PBS buffers. The washed bacteria is re-suspended in 1×PBS buffer and diluted to OD_{600} = 1.0.

Bacteria Labelling. The bacteria suspended in 1× PBS solution were incubated with **AIE-2Van** at varied concentrations. After 15 min incubation in dark, 10 μL portion of the bacteria suspension was spotted on polylysine pretreated glass slide and immobilized by the coverslips. The fluorescence images were immediately taken by confocal laser scanning microscope (CLSM) with signal collected above 560 nm upon excitation at 405 nm.

Naked-eye detection. For naked-eye detection, **AIE-2Van** is dispersed in 1× PBS solution at a concentration of 10 μM. To reduce the background signal originated from **AIE-2Van**, GO (1.7 mg/mL, 20 μL) was added into each tube of **AIE-2Van** solution. The bacteria suspension is then mixed with the AIE-2Van solution. The fluorescence image of the bacteria suspension is taken by a camera under the irradiation of a hand-handled UV-lamp (365 nm).

MIC activity. The MIC values were determined by a standard broth dilution methods. Vancomycin, AIEgen, and AIE-2Van were dissolved in DMSO or DMSO/water mixture to obtain a stock solution of 4 mg/mL. The stock solution was then diluted with LB solution into a series of sterile test t to obtain a final volume of 500 μL with different concentrations of 4, 8, 16, 32, 64, 128 μg/mL. 10 μL portion of bacteria suspension with OD_{600} value of 0.5 was then added into each tubes. The bacteria suspensions were then cultured at 37 °C for 24 h, and the OD_{600} was measured. The MIC values were determined as the lowest concentration that inhibited bacteria growth.

Antibacterial Assay. Colony-forming unit (CFU) counting method was applied to study the antibacterial effects of **AIE-2Van**. 90 μL of bacteria suspension (OD_{600} = 0.5) was transferred into a 96-well plate. **AIE-2Van** was then added into each well to achieve the final concentration of 0, 0.5, 1, 2, 4, 10 μM. The total volume of the bacteria suspension was kept at 100 μL and the incubation time is 15 minutes in dark. For the photodynamic inactivation (PDI) experiments, the bacteria suspensions in 96-well plate were exposed to white light irradiation (100 mW, 6 min). After irradiation, the bacterial suspensions were serially diluted 1×10^5 fold with 1× PBS buffer. 100 μL portion of the diluted bacterial suspension was spread on the solid LB agar plate and incubated at 37 °C for 16 h. The colonies formed

were counted and the percentages of dead bacteria were then determined by counting the remaining CFUs on the LB agar plate.

SEM Measurement. Based on the antimicrobial experiments, the concentration of **AIE-2Van** was determined to be 4 μM for SEM measurement. Followed by irradiation, bacteria were centrifuged at 5000 rpm for 5 minutes to remove 1×PBS. The bacteria were then suspended in and fixed by 2.5% glutaraldehyde for 2-3 hours at room temperature. The glutaraldehyde was removed by centrifuge, and the bacteria pellets were re-suspended in sterile water, and then 10 μL of bacteria suspension was spotted on to the SEM conducting paste. After natural drying in the air, the bacteria were dehydrated with a series of graded ethanol solution (30%, 50%, 70%, 80%, 90%, and 100% for 6 min). After drying overnight, the specimens were coated with platinum before SEM measurement.

Table S1. MIC values of vancomycin, AIEgen, and **AIE-2Van** compounds.

Compounds	*B. subtilis*	Van A	Van B
Van	5.2 μM	> 83.7 μM	> 83.7 μM
AIE	53.3 μM	> 213.3 μM	> 213.3 μM
AIE-2Van	4.4 μM	> 34.9 μM	> 34.9 μM

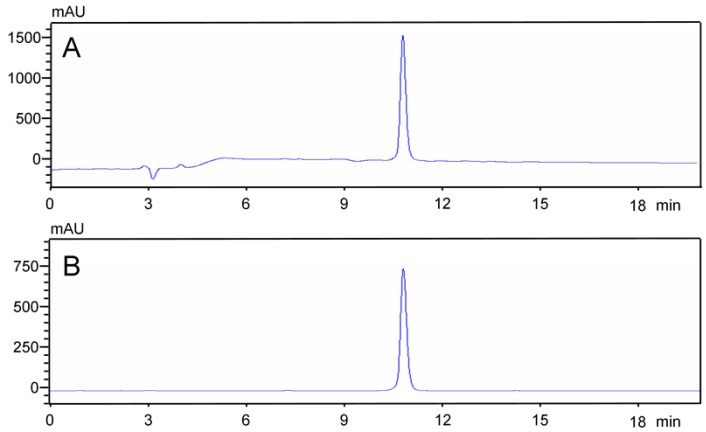

Fig. S1. HPLC spectra of the probe monitored at absorbance of 214 nm (A) or 430 nm (B).

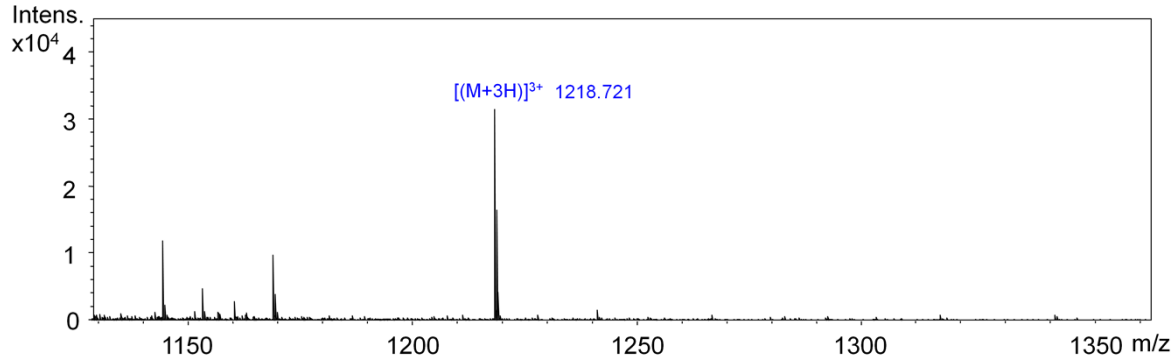

Fig. S2. Mass spectrum of the probe.

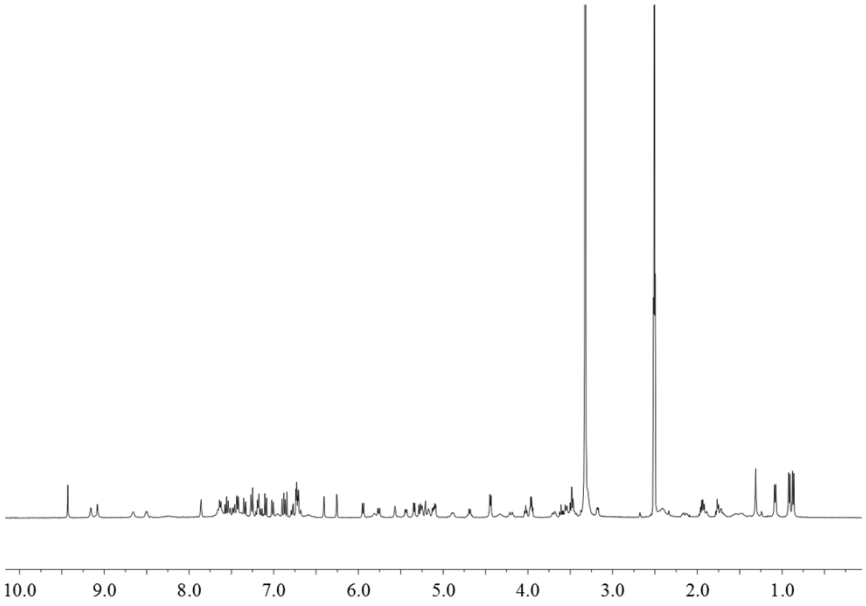

Figure S3. ^{1}H NMR spectrum of the probe.

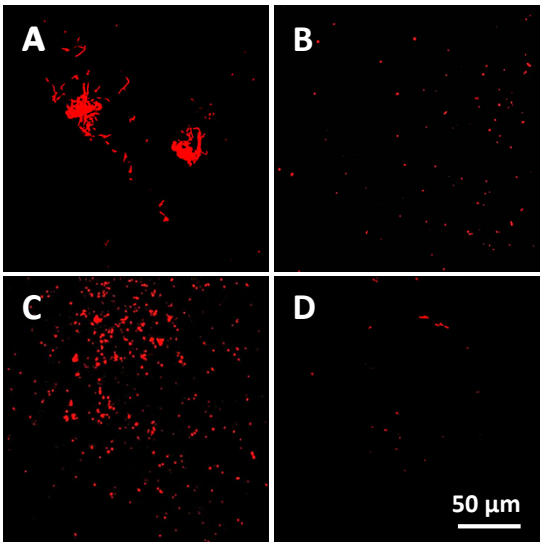

Fig. S4. CLSM images of A) *B. subtilis*, B) VanA, C) VanB, and D) *E. coli*, after incubation with AIE-2Van (4 µM) for 15 min. All the images share the same scale bar.

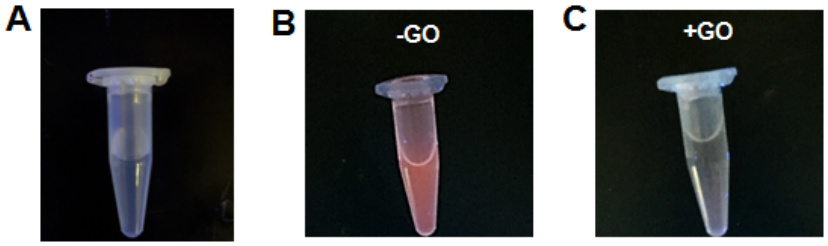

Fig. S5. Photographs of **AIE-2Van** (0.5 μM) in 1× PBS buffer (A) and **AIE-2Van** (10 μM) in 1× PBS buffer without GO (B) or with GO (C). The photographs were taken upon excitation by UV lamp with wavelength of 365 nm.

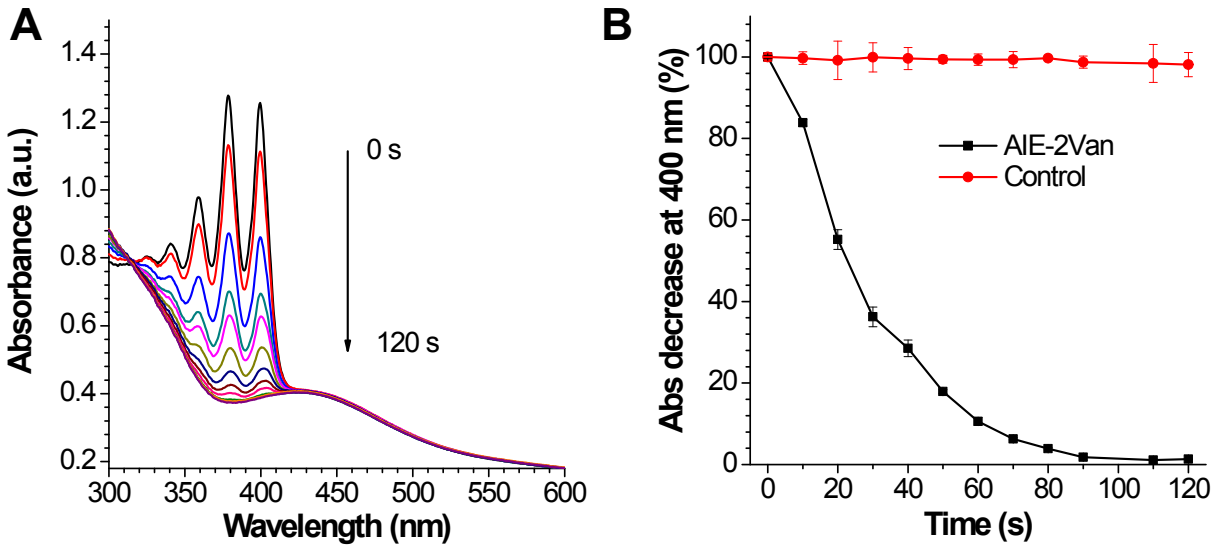

Fig. S6. A) Absorption spectra of ABDA in the presence of AIE-2Van and B. subtilis. B) The absorbance change of ABDA at 400 nm upon light irradiation in the absence and presence of AIE-2Van.

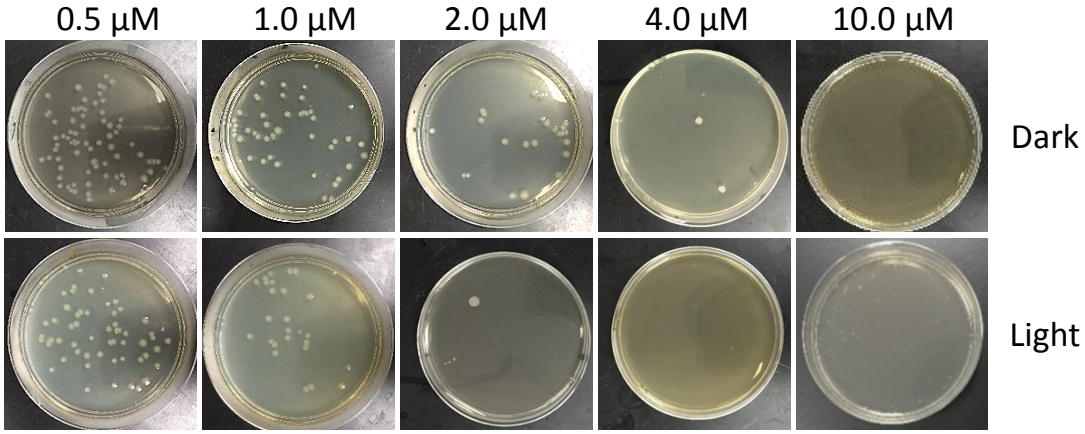

Fig. S7. Plate photographs for *B. subtilis* on LB agar plates supplemented with AIE-Van (A) or AIE-2Van (B) with and without white light irradiation (100 mW cm^{-2}) for 6 min then grew overnight.

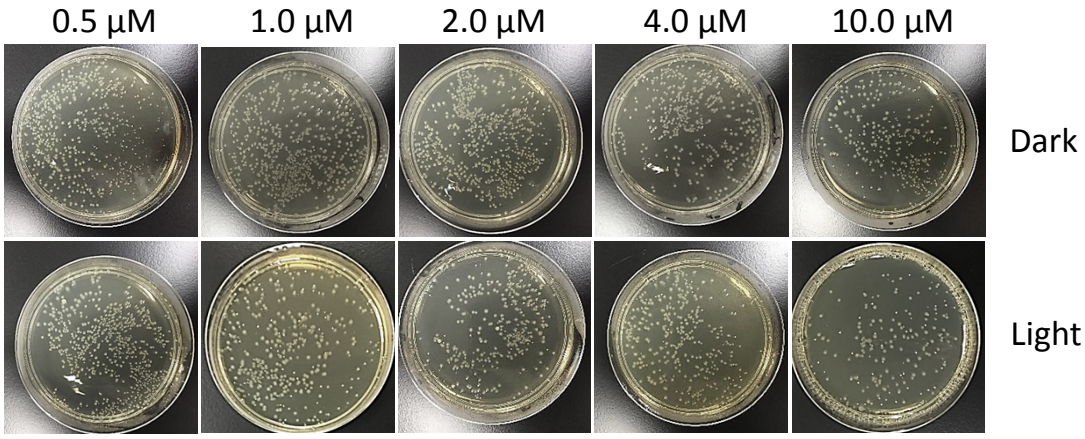

Fig. S8. Plate photographs for *E. coli* on LB agar plates supplemented with AIE-Van (A) or AIE-2Van (B) with and without white light irradiation (100 mW cm^{-2}) for 6 min then grew overnight.

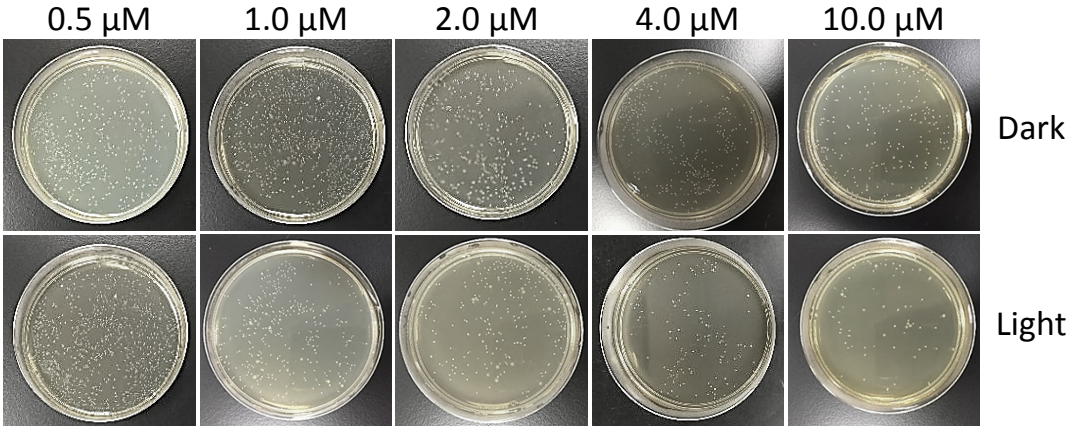

Fig. S9. Plate photographs for *Van A* on LB agar plates supplemented with AIE-Van (A) or AIE-2Van (B) with and without white light irradiation (100 mW cm^{-2}) for 6 min then grew overnight.

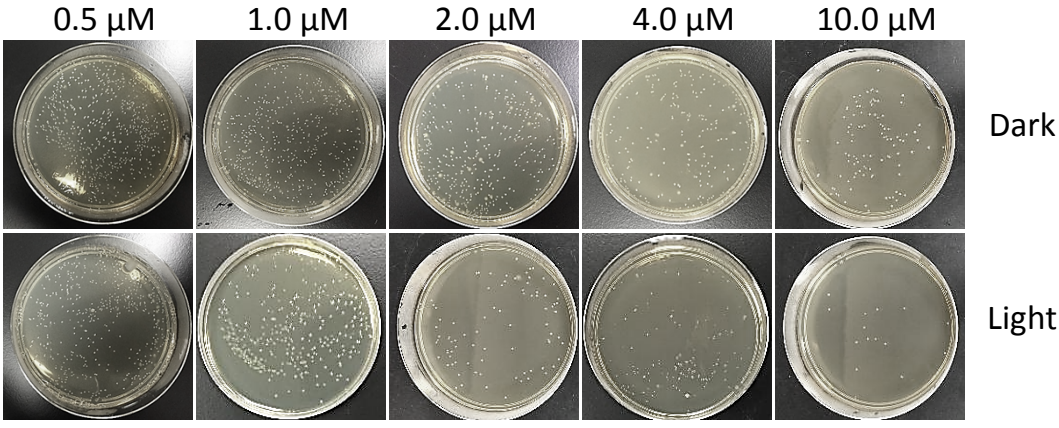

Fig. S10. Plate photographs for *Van B* on LB agar plates supplemented with AIE-Van (A) or AIE-2Van (B) with and without white light irradiation (100 mW cm^{-2}) for 6 min then grew overnight.

References:

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