Supporting Information

Synthesis of a New Cationic Non-conjugated Polymer for Discrimination of Microbial Pathogens

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EXPERIMENTAL SECTION

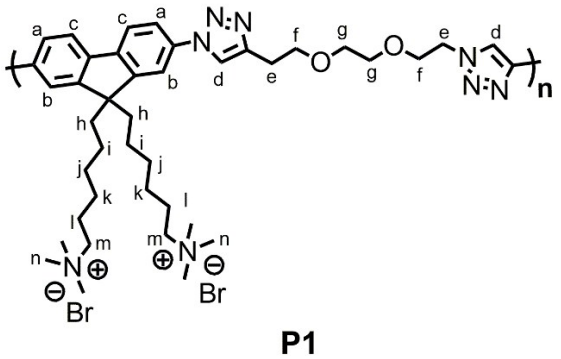
Materials. All chemicals were purchased from Aldrich, Acros, Alfa-Aesar or J&K Scientific LTD and used as received. All organic solvents were purchased from Beijing Chemical Works and used as received. 9H-fluorene was purchased from Amethyst Chemicals. Diazido triethyleneglycol was synthesized using the procedures as described in the literature.[1] 2,7-Diethylnyl-9,9-di(6'-bromohexyl)fluorine (1) were synthesized according to the procedures in the literature.[2] *Candida albicans* (*C. albicans*) ATCC 10231 and *Bacillus subtilis* (*B. subtilis*) were obtained from China General Microbiological Culture Collection Center. The Amp^r *Escherichia coli* (*E. coli*) was purchased from Beijing Bio-Med Technology Development Co., Ltd. Phosphate buffer saline (PBS) was purchased from Hyclone (Beijing, China), and water was purified with a Millipore filtration system.

Measurements. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Avance 400 MHz spectrometer. High resolution mass spectrum was recorded on a Thermo Fisher Scientific Exactive. The GPC measurement was performed on a Waters-410 system against polystyrene with DMF as eluent. UV-Vis absorption spectra were taken on a JASCO V-550 spectrophotometer. Fluorescence spectra were measured on a Hitachi F-4500 fluorometer equipped with a xenon lamp excitation source. The concentration of the microbial pathogens was determined by a JASCO V-550 spectrophotometer. The viability analysis for estimating toxicity was collected using a microplate reader (BIO-TEK Synergy HT, USA) at a wavelength of 600 nm. Zeta potentials were measured on a Nano ZS90 system. For phototoxicity test, the white light source was equipped with a metal halogen lamp (MVL-210, Mejiro Genossen, Japan). A radiometer (Photoelectric Instrument Factory of Beijing Normal University) was used to estimate the intensity of the incident beam. Phase contrast bright-field and fluorescence images were taken with a fluorescence microscope (Olympus 1×71) with a mercury lamp (100 W) as a light source.

Synthesis of compound 2: Condensed trimethylamine (2 mL) was added dropwise to a solution of compound 1 (270 mg, 0.50 mmol) in THF (5 mL) at 0 °C. The mixture was allowed to warm to 40 °C. The precipitate was redissolved by the addition of methanol (5 mL). After the mixture was cooled to 0 °C, additional trimethylamine (2 mL) was added. The mixture was stirred at 40 °C for 24 h. After removal of the solvent, acetone was added to precipitate 2 (329 mg, 100%) as yellow powders. ^{1}H-NMR (400 MHz, CD_3OD-d_4, ppm): δ 7.75 (d, 2H), 7.53 (s, 2H), 7.47 (d, 2H), 3.60 (s, 2H), 3.18 (m, 4H), 3.04 (s, 18H), 2.08 (m, 4H), 1.55 (m, 4H), 1.16 (m, 8H), 0.58 (m. 4H). ^{13}C-NMR (100 MHz,

CD_3OD-d_4, ppm): δ 152.1, 142.4, 132.5, 127.5, 122.8, 121.3, 85.0, 79.4, 67.7, 56.4, 53.4, 40.6, 29.9, 26.5, 24.4, 23.4. HRMS (ESI) m/z: $[M]^{2+}$ calcd 249.198151, found 249.198271.

Synthesis of polymer P1: A two-necked flask was charged with diazido triethyleneglycol (20.0 mg, 0.10 mmol), compound 2 (65.8 mg, 0.10 mmol), sodium ascorbate (198.1 mg, 1.00 mmol), 1 mL of water and 1 mL of DMSO. The mixture was stirred under argon for 2 h at room temperature. After the mixture was completely dissolved, a solution of $CuSO_4$ (32.0 mg, 0.20 mmol) in water (1 mL) was added dropwise to the reaction mixture. Then the reaction was kept at room temperature under argon atmosphere for 2 days. After the reaction was completed, the product was purified by dialysis against water using 3500 Da molecular weight cutoff dialysis membrane for 3 days to yield 8.2 mg of P1 as a dark yellow solid. Yield: 9.9%. The fluorescence quantum efficiency is 2% for polymer P1. ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): 8.78 (br, $2H_d$), 8.01 (br, $2H_c$), 7.85 (br, $4H_{a,b}$), 4.58 (br, $4H_e$), 3.88 (br, $4H_f$), 3.57 (br, $4H_g$), 3.12 (br, $4H_m$), 2.91 (br, $18H_n$), 2.07 (br, $4H_h$),1.36 (m, $4H_l$), 1.01 (br, $8H_{i,k}$), 0.55 (br, $4H_j$). GPC analysis (Mn: 5846, Mw: 5921, PDI: 1.01).



P1

Figure S1. The structure of P1.

Preparation of bacteria and fungi solutions: A single colony of *B. subtilis* (Gram-positive) on a solid Beef-extract Peptone Yeast-extract (BPY) agar plate was transferred to 10 mL of liquid BPY culture medium and grown at 37 °C for 8 h. 2mL *B. subtilis* were harvested by centrifuging (8000 rpm for 2 min) and washing with phosphate buffer saline (PBS, 10 mM, pH = 7.4) three times. The supernatant was discarded and the remaining *B. subtilis* was resuspended in PBS, and diluted to an optical density of 1.0 at 600 nm (OD_{600} = 1.0). As for Amp^r *E. coli* (Gram-negative), except that the culture medium was replaced by Luria Broth (LB, supplemented with 100 mg/mL ampicillin), other experimental conditions and operations were totally the same as that of *B. subtilis*. A single colony of *C. albicans* (fungi) on a solid Yeast-extract Peptone Dextrose (YPD) agar plate was transferred to 10 mL of liquid YPD culture medium and grown at 30 °C for 10 h. The following operations were totally the same as those of *B. subtilis*, except that the optical density at 600 nm was replaced by 2.0.

Zeta potential measurements: *B. subtilis*, *E. coli* and *C. albicans* in PBS (10 mM, pH= 7.4) were incubated separately with different concentrations of P1 (5.0 μM, 10.0 μM,

30.0 μM and 50.0 μM in RUs, respectively) for 20 min at 37 °C for *E. coli* and *B. subtilis* and 30 °C for *C. albicans*, respectively. And then the unbound P1 was removed by centrifugation (8000 rpm, 2 min). The obtained pellets were washed once and resuspended with 1 mL of PBS (10 mM, pH= 7.4) and the suspensions were kept on ice for zeta potential measurements. As negative controls, untreated bacteria or fungi (without P1) were also disposed under exactly the same conditions.

Fluorescence microscopy measurements: Suspensions of *B. subtilis* (1 mL, OD_{600} = 1.0), *E. coli* (1 mL, OD_{600} = 1.0) and *C. albicans* (1 mL, OD_{600} = 2.0) in PBS (10 mM, pH= 7.4) were incubated separately with different concentrations of P1 (5.0 μM, 10.0 μM, 30.0 μM and 50.0 μM in RUs, respectively) for 20 min at 37 °C for *E. coli* and *B. subtilis* and 30 °C for *C. albicans*, respectively. And then the unbound P1 was removed by centrifugation (8000 rpm, 2 min). The obtained pellets were washed once and resuspended with 1 mL of PBS (10 mM, pH= 7.4) and the suspensions were kept on ice for fluorescence microscopy measurements. As negative controls, untreated bacteria or fungi (without P1) were also disposed under exactly the same conditions. Solutions (10 μL) of *B. subtilis*, *E. coli* and *C. albicans* incubated without or with P1 were added to clean glass slides followed by slightly covering coverslips for immobilization, and then photographs were taken. The phase contrast images and the fluorescence images were taken via fluorescence microscopy with the exposure time of 100 ms. The false color was blue for Channel P1. The type of light filter was D380/30 nm exciter, 420 nm beamsplitter, and D460/50 nm emitter. Magnification of the object lens was 100×.

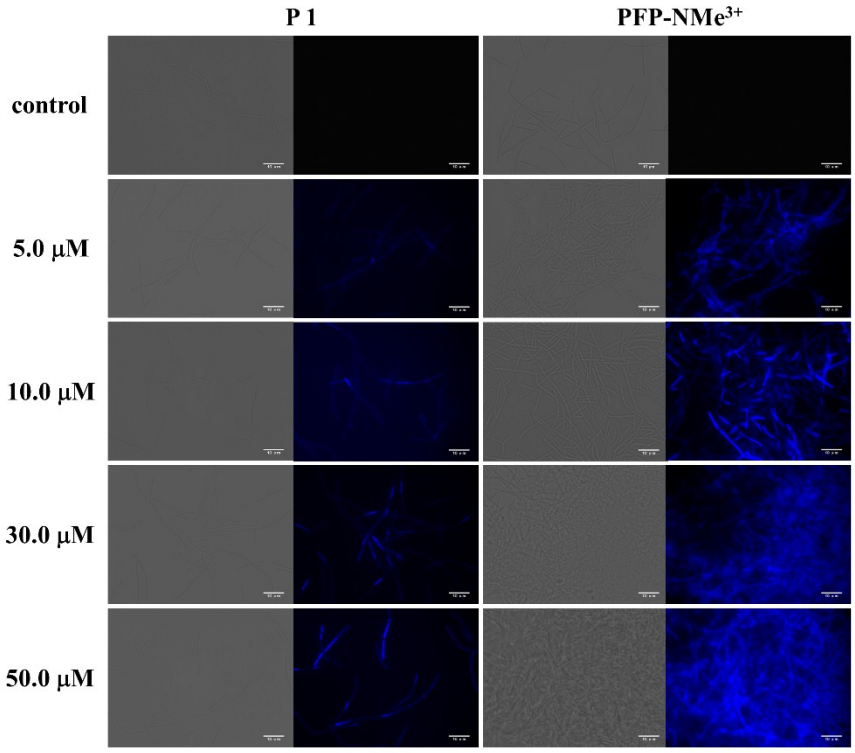


Figure S2. The interactions between *B. subtilis* and various concentrations of P1/PFP in PBS (10 mM). Scale bar is 10 um.

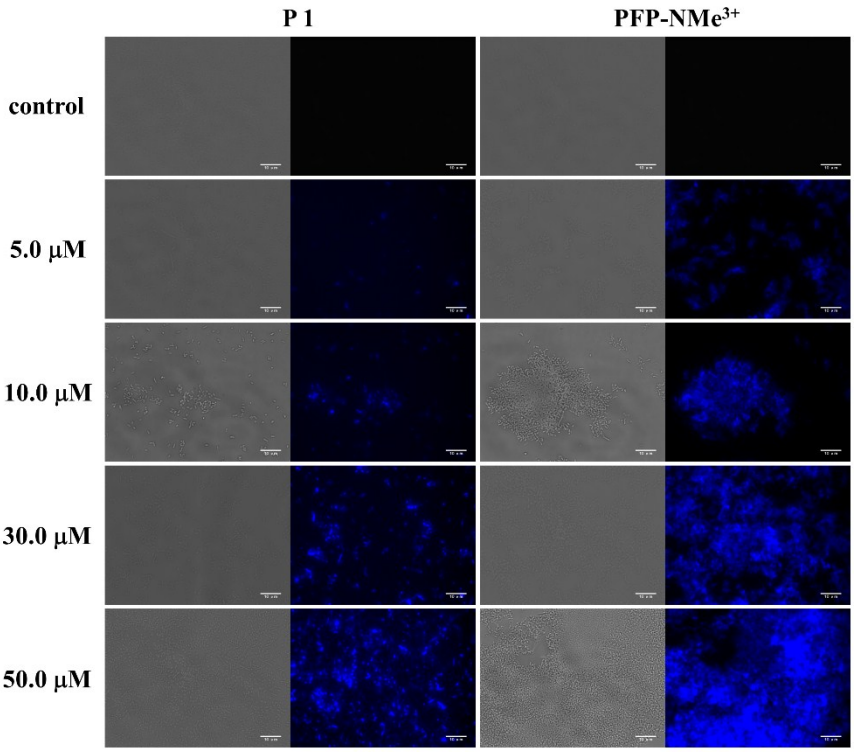


Figure S3. The interactions between *E. coli* and various concentrations of P1/PFP in PBS (10 mM). Scale bar is 10 um.

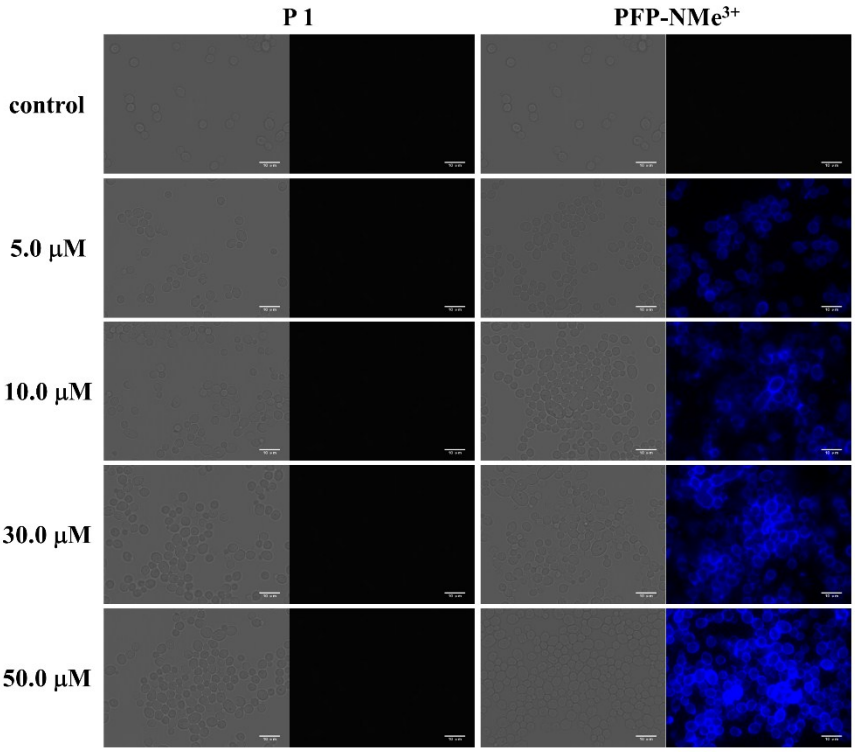


Figure S4. The interactions between *C. albicans* and various concentrations of P1/PFP in PBS (10 mM). Scale bar is 10 um.

The measurements of viability with P1 for microbes: Microbes were seeded in 96-well plates at a density of 2×10^6 cells/well in all the experiments. The microbes used here were *B. subtilis*, *E. coli* and *C. albicans*. *B. subtilis* and *E. coli* were incubated separately with different concentrations of P1 at 37 °C for 1-10 h, for *C. albicans*, incubated at 30 °C for 1-10 h. After shaking the plate for 2 min, the absorbance at 600 nm of each well was read by a microplate reader. The results were plotted as "viability". The viability (%) was calculated according to the following equation:

$$I\ (\%) = \{(C-B)/\ (A-B)\} \times 100\%$$

where A is the absorbance of the microbes control (without adding P1, other operations were identical to the experiment group), B is the absorbance of the culture medium control (only added equivalent culture medium used in experiment group, other operations were identical to the experiment group) and C is the absorbance of the experiment group.

The measurements of OD_{600} with P1 for microbes: Microbes were seeded in 96-well plates at a density of 2×10^6 cells/well in all the experiments. The microbes used here were *B. subtilis*, *E. coli* and *C. albicans*. *B. subtilis* and *E. coli* were incubated separately with different concentrations of P1 at 37 °C for 1-10 h, for *C. albicans*, incubated at 30 °C for 1-10 h. After shaking the plate for 2 min, the absorbance at 600 nm of each well was read every hour by a microplate reader.

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[2] He, F.; Ren, X.; Shen, X.; Xu, Q. Water-Soluble Conjugated Polymers for Amplification of One and Two-photon Properties of Photosensitizers. *Macromolecules* **2011**, *44*, 5373-5380.

[3] Bourré, L.; Thibaut, S.; Briffaud, A.; Rousset, N.; Eléouet, S.; Lajat, Y.; Patrice, T. Indirect Detection of Photosensitizer ex Vivo. *J. Photochem. Photobiol. B.* **2002**, *67*, 23–31.