Support information

Photosensitizer-AgNPs composite with pathogen selective recognition ability and enhanced photodynamic efficiency

Jiaqi Zhang[a], Zhentan Lu*[a], Zhenguo Yu, Weibing Zhong[b], Haiqing Jiang[a], Qinghua Zhao[a], Fei Li[a] , Xinge Zhang[c] and Dong Wang*[a,b]

a. Hubei Key Laboratory of Advanced Textile Materials & Application,College of Materials Science and Engineering, Wuhan Textile University, Wuhan 430200, China.

E-mail: luzt2015@126.com; wangdon08@126.com.

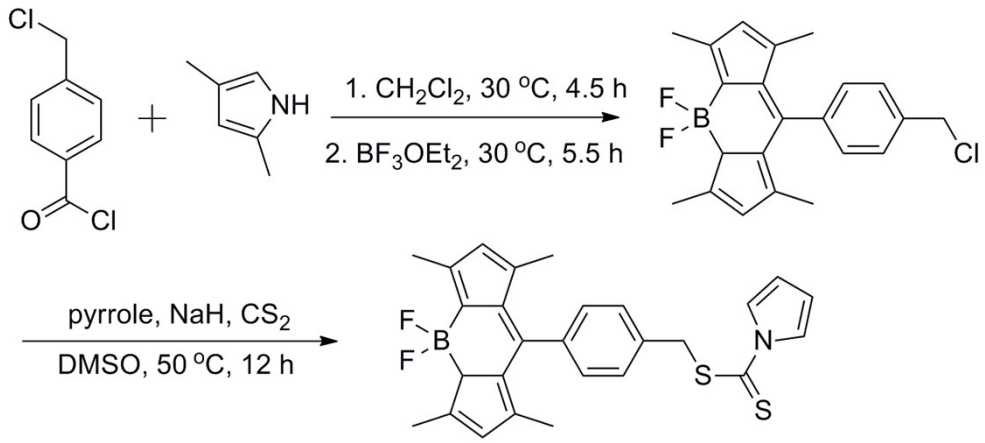
b. National Engineering Research Center for Dyeing and Finishing of Textiles,College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China

c. Key Laboratory of Functional Polymer Materials Ministry of Education, Institute of Polymer Chemistry, Nankai University, 94# Weijin Road, Tianjin 300071, China.

1. The synthesis BODIPY-based RAFT chain-transfer agent

P-(Chloromethyl) benzoyl chloride (1.5 g, 8 mmol) was added dropwise to a stirred solution of freshly distilled 2,4-dimethylpyrrole (2.2 mL) in dichloromethane (90 mL) at room temperature and under N_2, and the mixture was heated to 30 °C with stirring for 4.5 h. Then, triethylamine (3.88 g, 38 mmol) were added to the mixture, the mixture was stirred at room temperature for 30 min under N_2, and boron trifuoride diethyl etherate (7.82 g, 55 mmol) was then added. After heating to 30 °C for 5.5 h, the subsequent workup yielded a residue that was purified by silica gel column chromatography eluted with ethyl acetate and petroleum ether (v/v = 1:20) to give a red solid with 36% yield.

Pyrrole (0.17 mL, 2.38 mmol) was added dropwise to a stirred suspension of sodium hydride (57.1 mg, 2.38 mmol) in dimethyl sulfoxide (50mL). On completion of addition, the resulting brown solution was stirred at room temperature for 30 min before the addition of carbon disulfide (0.14 mL, 2.38 mmol). The solution was allowed to stir at room temperature for a further 30min, and BODIPY-Ar-Cl (1.02 g, 2.38 mmol) was added. Water (500 mL) was added after the reaction mixture was stirred at 50 °C for 12 h, followed by $CHCl_3$ (30 mL). The organic layer was separated, and the aqueous layer was extracted with $CHCl_3$ (2×30 mL). The combined extracts were dried with anhydride magnesium sulfate and filtered, and the solvent was removed. The crude product was purified by gel column chromatography eluted with ethyl acetate and petroleum ether (v/v = 1:10) to give a red solid with 43% yield. We named the solid as BODIPY-RA.



Scheme S1. Synthesis of BODIPY-RAFT.

2. Synthesis BODIPY-functionalized glycopolymer

Synthesis of pAcGEMA was carried out by RAFT at 70 °C in 1,4-dioxance solution (50 wt%) for 18 h. In order to introduce iodine atom into the BODIPY molecular, excess N-iodosuccinimide was used in anhydrous dichloromethane for iodination. The final product was a deep red solid of pAcGEMA-I. Finally, the polymer was deacetylated using hydrazine in DMSO for 24 h at 25 °C under nitrogen, and the resultant solution was dialyzed against water (MWCO 3500) for 3 days before lyophilization to get a red solid pGEMA-I. pGEMA-I were characterized with ^{1}H NMR.

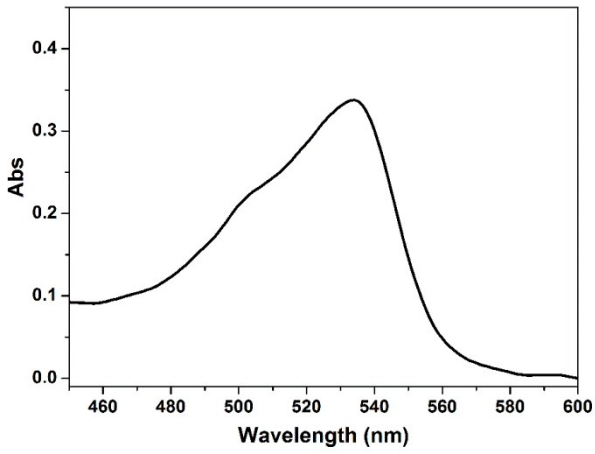


Fig. S1. The absorption spectrum of pGEMA-I (5 pmol/mL)

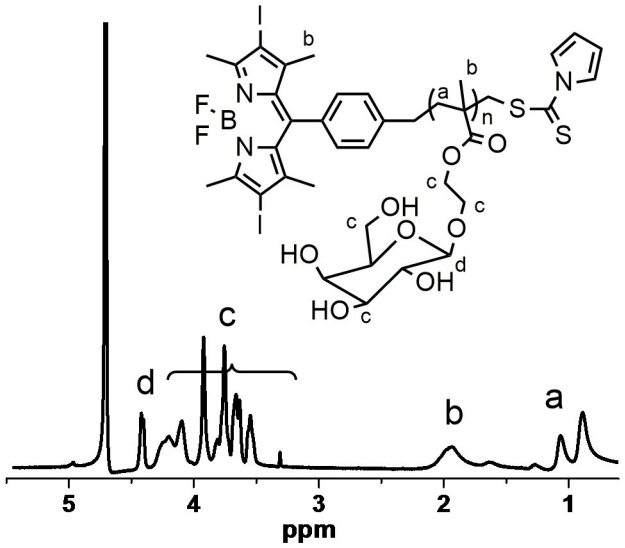


Fig. S2. ^{1}H NMR of pGEMA-2I (D_2O as solvent).

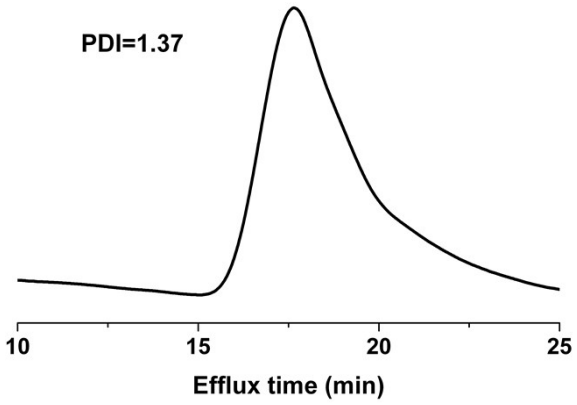


Fig. S3. GPC profile of pAcGEMA. (THF as eluent, polystyrene as reference)

3. Preparation of BODIPY-I@AgNPs

100 μL of 10 mg/mL $AgNO_3$ and 100 μL of 10 mg/mL copolymer were mixed with 5.0 mL of deionized under vigorous stirring (600 r/min) in an ice-water bath. Then, excess fresh $NaBH_4$ was rapidly added under stirring for about 30 min to get BODIPY-I@AgNPs solution. The BODIPY-I@AgNPs solution was dialyzed (8-14 kDa M.W. cuto□) against ultrapure water for 24 h and stored at 4 °C.

We had detected the dialysis solution EDX and UV spectra, Ag and pGEMA-I

were not detected. So loading of the photosensitizer (BODYPY) on the BODIPY-I@AgNPs (61.2%) was depended the feed ratio.

4. Cell selectivity of BODIPY-I@AgNPs

The cell selectivity of the BODIPY-I@AgNPs was visualized using a confocal laser scanning microscope (CLSM). The mixture of BODIPY@AgNPs (0.5 nmol/mL, without iodine) and bacterial suspension (OD600=0.5) was incubated under dark at 37 ^{o}C for 30 min. Then, the suspension was washed with PBS three times. The fluorescence photographs of bacteria were obtained by CLSM with argon laser, the maximum excitation and emission wavelength for pGEMA was 488 and 506 nm. A number of 1.0×10^5 of cells were seeded on glass bottom culture dishes (12 mm) and incubated for 24 h. Then, the cell culture medium was removed, and 200 μL of the sample solution (0.5 nmol/mL) was added into the dish for further incubation. At the predetermined time (30 min), the cell culture medium was removed, and the cells were rinsed with PBS three times and fixed with 200 μL of 4% paraformaldehyde in PBS for 10 min, and further washed with PBS three times. The cells were incubated with 200 μL of 10% DAPI solution in PBS for 20 min to stain the cell nucleus, and then the solution was removed and washed with PBS three times. The fluorescence photographs of stained cells were obtained by CLSM with argon laser; the maximum excitation and emission wavelength for the sample were 488 and 506 nm, and the maximum excitation and emission wavelength for DAPI were 360 and 488 nm, respectively.

5. The detection of generation rate of $^1O_2$

To detect the generation rate of $^1O_2$ of pGEMA-I and BODIPY@AgNPs in aqueous system, a simple test was performed according to the published method. P-nitrosodimethylaniline (RNO) and histidine were used as $^1O_2$ scavenger. BODIPY photosensitizer had absorption spectrum between 450-580 nm and displayed maxima wavelength at 535 nm, the absorption spectrum in the range of sunlight visible

spectrum (400-800 nm), and so simulated sunlight was used as the illumination source. RON does not react with $^1O_2$ directly; however, it can react with the product formed by the reaction of $^1O_2$ with histidine reducing an absorption loss at 440 nm. A solution of neutral aqueous buffers containing RNO (5 mg/mL), histidine (100 mg/mL) and PDT agents (5 nmol/mL) under investigation was prepared, this solution was transferred into a glass cuvette, placed in the spectrophotometer and irradiated under the white light (1.5 mW/cm^2, 400-800 nm) at room temperature. The rate of $^1O_2$ production was determined by measuring the decrease of RNO absorbance at 440 nm at different time intervals. Irradiation was also carried out on a RNO solution in the absence of photosensitizer (negative control).

6. Bacterial growth inhibitory assay

The effect of the PDT agent concentration on the antibacterial activity was also studied using *P. aeruginosa* and *S. aureus* as the model bacteria. The bacterial suspension was further diluted (1 in 100) to give a working concentration of approximately 10^6-10^7 CFU/mL. BODIPY-I@AgNPs and pGEMA-I were dissolved in deionized water to obtain various concentrations. The PDT agent solutions (1 mL) were added to the bacterial suspension (1 mL). The mixtures were incubated under white light (400-800 nm, 25 mW/cm^2) at 37 ^{o}C for 15 min and then incubated at 37 ^{o}C for a further 12 h in the dark. Control samples incubated without illumination were used to evaluate the antibacterial activity under dark conditions. The OD600 value of the solution was also used to determine the amount of bacteria. Three independent experiments were carried out.

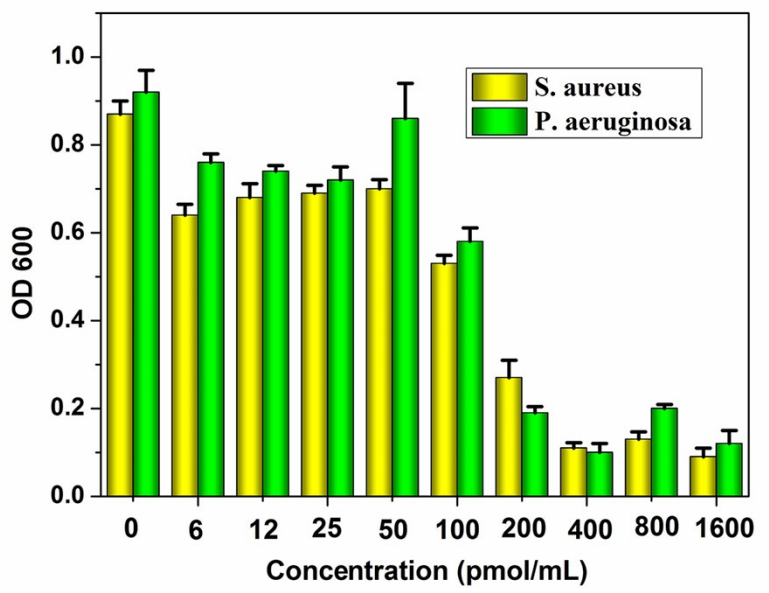


Fig. S4. Antibacterial activity of pGEMA-I with different concentration under illumination. The sample without copolymer was set as negative control.

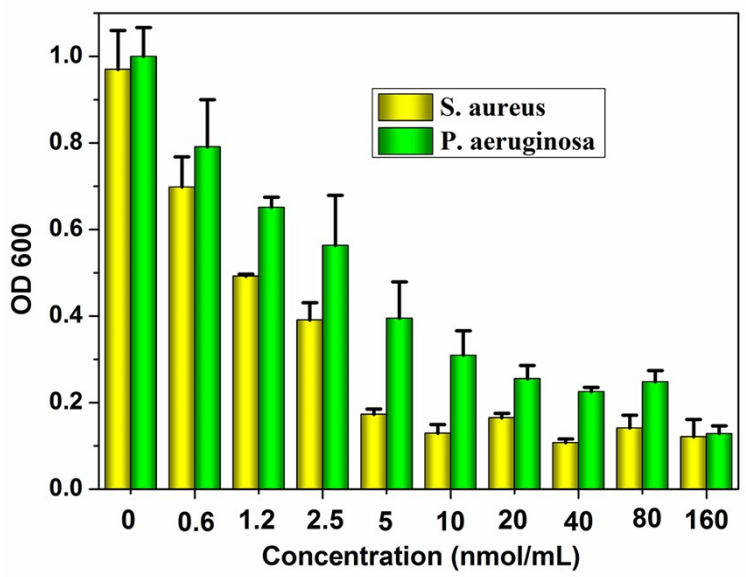


Fig. S5. Antibacterial activity of BODIPY-I@AgNPs with different concentration under dark. The sample without AgNPs was set as negative control.

7. Hemolysis of BODIPY-I@AgNPs

The hemolysis of BODIPY-I@AgNPs was examined by incubation with human blood based on a previously published method. Fresh human blood (5 mL) was collected from a healthy donor in Tianjin Medical University. Erythrocytes were separated by centrifugation at 5000 r/min for 5 min, washed three times with Tris

buffer (10 mM Tris, 150 mM NaCl, pH 7.2) and finally diluted with an erythrocyte stock solution (5%). The solution was mixed with different concentrations of the macromolecular photosensitizer solution (0.5, 5, 10, and 20 nmol/mL) and incubated for 1 h at 37 °C. The samples were then centrifuged at 5000 r/min for 5 min. The hemolytic activity was determined by absorption at 540 nm using a UV spectrophotometer. Tris buffer was used as a negative control and 0.1% Triton X-100 in Tris buffer was used as a positive control. The hemolytic percentage (hemolysis%) was calculated from the following equation: hemolysis% = [(Am−An)/(Ap−An)] × 100%, where Am is the absorbance of the cells and macromolecular photosensitizer, An is the absorbance of the negative control and Ap is the absorbance of the positive control.

8. Cytotoxicity of the BODIPY-I@AgNPs

The cells (200 μL) were seeded in 96-well plate at a density of 1.0×10^4 cells per well in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum and incubated for 24 h at 37 ^{o}C in 5% CO_2. The solution of BODIPY-I@AgNPs was diluted with DMEM to obtain predetermined concentrations (400, 200, 100, 50 and 25 pmol/mL), respectively. The medium in the 96-well was replaced with the BODIPY-I@AgNPs solution (200 μL). And, then, 20 μL MTT was added into each well incubated for further 4 h when the cells were cultured for 24 and 48 h. Then the medium was completely removed, and 150 μL dimethylsulfoxide (DMSO) was added to each well to dissolve the formazan crystals. The plate was placed at 37 ^{o}C for 10 min, and then the optical density was read on a microplate reader at 490 nm in

triplicate. The cells without NPs were used as a control and their viability was set at 100%.

These samples were illuminated under white light (400–800 nm, 25 mW/cm^2) for 15 min, and then were cultured for another 24 h under dark to evaluate the light-cytotoxicity of BODIPY-I@AgNPs to cells.

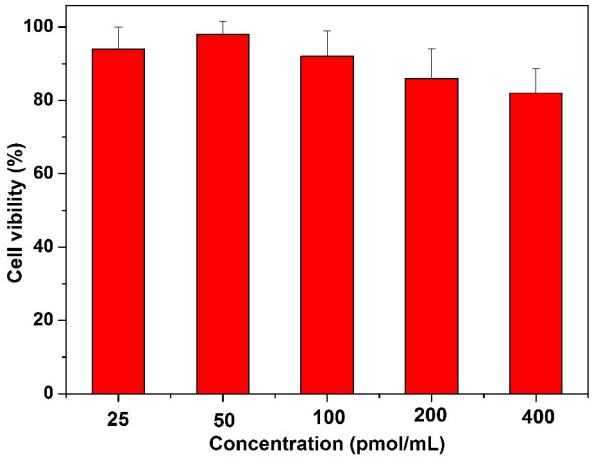


Fig. S6 Cell viability of NIH3T3 cells after the treatment with BODIPY-I@AgNPs under illumination.

9. Preparation of bacteria samples for SEM

The bacteria samples were fixed with 2.5% glutaraldehyde overnight on a clean glass slide (washed with NaOH–ethanol solution and water). Then, the bacteria were further washed with water, dehydrated using graded ethanol solutions (30, 50, 70, 90, 95, 100% v/v in water) and dried in a freeze dryer. The samples were coated with platinum and observed using a field-emission scanning electron microscope (Shimadzu SS-550).