Supporting Information

Materials and Measurements

Potassium 2,7-dibromo-9,9-bis(3'-sulfonatopropyl) fluorene, 2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-bis[3'-(dimethylamino) propyl] fluorene, and poly{9,9-di[3'-(1-ethyl-1,1-dimethylammonio) propyl]-2,7-fluorenyl-alt-1,4 -phenylene dibromide} (PFN) were synthesized according to previous methods [1] . 4,7-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,1,3-benzothiadiazole and 4,7-bis(5-bromo-2-thienyl)-2,1,3-benzothiadiazole were purchased from Beijing Allmers Chemical Science & Technology. DiI, paraformaldehyde, and WST-1, i.e. 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2, 4-disulfophenyl)-2H-tetrazolium monosodium salt, kits were obtained from Beyotime Institute of Biotechnology (Haimen, China). Tetrakis-(triphenyl-phosphine) -palladium (0) and all other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as received. Liver cancer cell line (Bel-7402) was obtained from Shanghai Institute of Cell Biology (Shanghai, China). All cell culture materials were purchased from Invitrogen.

^{1}H-NMR spectra were collected on a Bruker DRX-300 spectrometer. Ultraviolet-visible spectra and fluorescence spectra were collected on a UV-1800 PC spectrophotometer and a RF-5301 PC fluorescence spectrometer, respectively. The excitation wavelengths of PFN, BtPFS, and TbtPFN are 369 nm, 430 nm, and 530 nm, respectively.

Synthesis of BtPFS

Potassium 2, 7-dibromo-9, 9-bis (3'-sulfonatopropyl) fluorene (249mg, 0.386mmol) and 4,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,1,3-benzothiadiazole (150mg, 0.386mmol) were dissolved in a solution containing 50 mL 2 mol/L potassium carbonate and 25 mL N,N-dimethylformamide. Tetrakis-(triphenylphosphine) palladium (0) (50mg) was added rapidly into the degassed solution under argon atmosphere. The reaction continued under 85~90 ^{o}C for 48 h. After the reaction, the solution was poured into a beaker with 500 mL acetone. The precipitation was collect by centrifugation and washed by acetone (3×5 mL). The product was dissolved and dialyzed in distilled water using a membrane with the cutoff molecular weight of 8000-10000

g/mol for three days. The precipitation was removed after centrifugation. The final product was gathered by rotary evaporation to afford 105 mg orange powder (BtPFS). The yield was 49%.

^{1}H-NMR (300 MHz, D_2O-DMSO). δ (ppm): 8.2-7.5 (m, 8H, aromatic rings), 2.6-2.4 (m, 4H, fluorene -CH_2), 2.4-2.1 (m, 4H, -CH_2SO_3K), 1.2-0.9 (m, 4H, -CCH_2C-).

Synthesis of TbtPFN

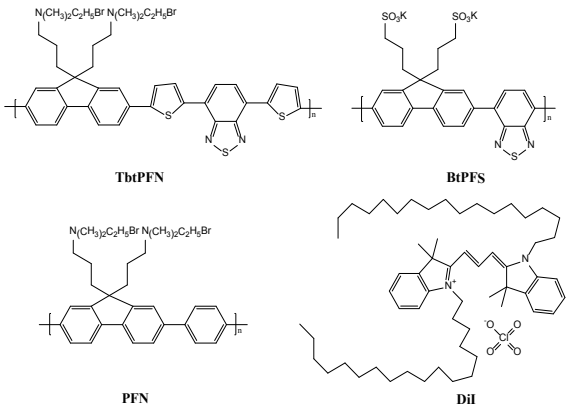
2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9-bis[3'-(dimethylamino) propyl] fluorene (100 mg, 0.170 mmol) and 4,7-bis(5-bromo-2-thienyl)-2,1,3 -benzothiadiazole (77.5 mg, 0.170 mmol) were dissolved in 40 mL toluene and 20 mL 2 mol/L potassium carbonate aqueous solution. Tetrakis-(triphenyl phosphine) palladium (0) （55 mg）was added quickly into the degassed solution under argon atmosphere. The reaction continued under 85~90 ^oC for 48 h. After the reaction, the solution was extracted by chloroform (3×15 mL) and washed by saturated brine (3×10 mL) and water (3×10 mL). The extract was dried by anhydrous magnesium sulfate. Crude product was obtained from rotary evaporation to remove solvent. Red-brown powder (TbtPF, 62 mg) was collected after washed by acetone and dried by vacuum evaporation to bear the 58% yield.

^{1}H-NMR (300 MHz, $CDCl_3$). δ (ppm): 8.2-7.8, 7.7-7.5 (m, 12H, aromatic rings), 2.3-1.9 (m, 20H, -NCH_2, -NCH_3, -CH_2-fluorene), 1.0-0.8 (m, 4H, -CCH_2C-).

Red-brown powder (TbtPF, 40 mg) was dissolved in 60 mL tetrahydrofuran and 15 mL dimethyl sulfoxide in a 250 mL three-necked bottle. Bromoethane (2.9 g, 27 mmol) was added into the solution under magnetic stirring. The reaction continued under 50 ^oC for 5 days, and distilled water was added to dissolve the precipitation. Red-brown solid (TbtPFN, 39 mg) was obtained after precipitation by acetone and vacuum evaporation with 68% yield.

^{1}H-NMR (300 MHz, DMSO-d_6). δ (ppm): 8.3-7.8 (m, 12H, aromatic rings), 3.3-3.1 (m, 8H, -NCH_2), 3.1-2.7 (m, 16H, -NCH_3, -CH_2-fluorene), 2.5 (m, 4H, -CCH_2C-), 2.2-2.0 (m, unquaternized –$NCH_{x=2-3}$), 1.0-0.9 (m, 6H, -CCH_3).

The molecular weights of the purified conjugated polymers were determined by GPC (calibrated by PEG standard). The number-average molecular weights of PFN, BtPFS, and TbtPFN are 1.1×10^4, 2.3×10^4, and 2.9×10^4, respectively.



Scheme S1. Chemical structures of the conjugated polymers and the lipophilic tracer DiI.

Cell Culture

Bel-7402 cells were cultured in DMEM (Dulbecco's Modified Eagle Medium) containing 10% fetal bovine serum and 1% penicillin streptomycin at 37 ^{o}C in a humidified environment containing 5% CO_2. Before experiment, the cells were pre-cultured until confluence was reached.

Fluorescence Imaging

Bel-7402 cells were seeded onto coverslips in a 6-well plate at an intensity of 1×10^5 cells per well and the plate was washed with PBS (phosphate buffered saline, pH 7.4). Then cell culture mediums with the conjugated polymer (50 μmol/L) were added into the wells separately. After 4 h incubation at 37^{o}C, the medium was removed from each well and the wells were washed thrice with PBS. The cells were fixed with fresh 4% paraformaldehyde for 15 min at room temperature and the fixed cells were finally washed thrice with PBS. The fluorescence imaging was performed on an Olympus IX71 fluorescence microscope. The excitation wavelengths of the cells incubated with PFN, BtPFS, and TbtPFN were 405 nm, 405 nm, and 488 nm, respectively. The power of the Hg-Lamp was 600 w, and the exposure time to record the images was all 600 ms.

Cytotoxicity Test

The WST-1 assay was employed to evaluate the effect of the conjugated polymers on the cell viability. Bel-7402 cells in 200 μL culture medium were seeded in a 96-well plate at an intensity of 1×10^4 cells per well. After overnight incubation at 37^{o}C, the medium was replaced with 200 μL fresh medium containing the conjugated polymers with various concentrations. One row of the 96-well plate was used as control. After incubation for 8 h or 24 h in a humidified incubator with 5% CO_2 at 37 ^{o}C, 20 μL WST-1 were added to each well and further incubated for another 2 h. The absorbance at 450 nm was detected employing a microplate reader (Thermo Electron Corporation)

and results were shown as the average cell viability ([(OD_{treat}-OD_{blank})/($OD_{control}$-OD_{blank}) ×100%]) of triplicate wells, where OD is the optical density.

Photobleaching Test

An ultraviolet low pressure mercury lamp (the power was 60 w) was employed to irradiate the aqueous solutions of the samples (10^{-5} mol/L) or the co-incubated cells. The illumination area of each sample well was 3 cm^2, and the fluence rate was 20 w/cm^2.

Scheme S2. Sketch of the interaction between BtPFS and the cell membrane.

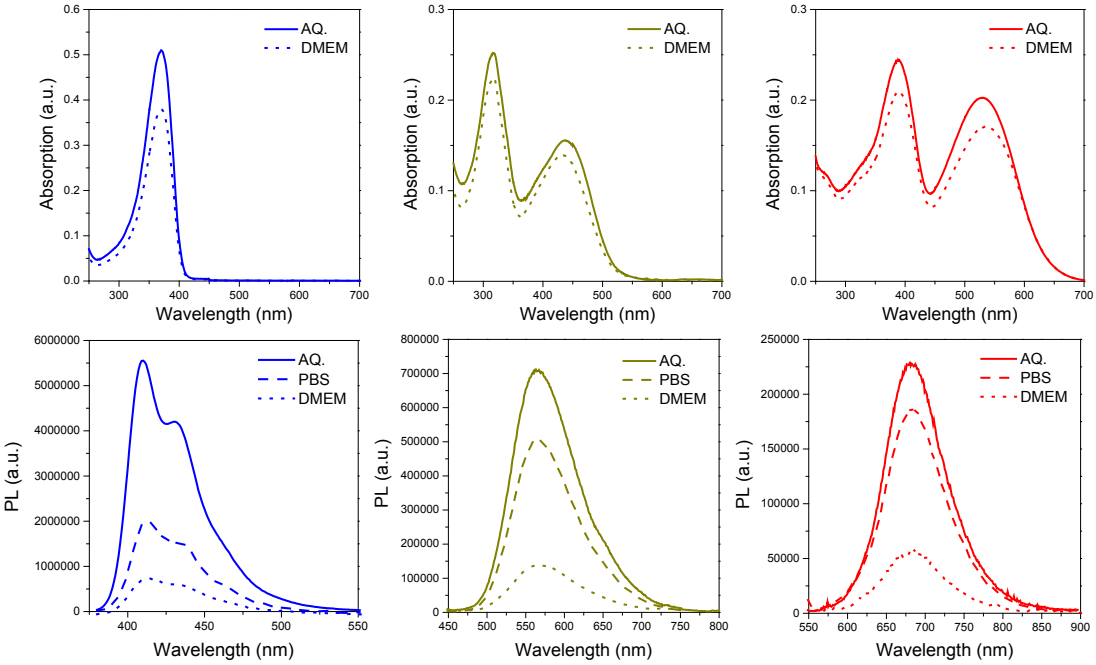


Figure S1. Upper row: Absorption spectra of PFN (left), BtPFS (middle), and TbtPFN (right) in aqueous solution (AQ.) and DMEM with the same concentration (10^{-5} mol/L). Bottom row: Photoluminescence spectra of PFN (left), BtPFS (middle), and TbtPFN (right) in aqueous solution, PBS, and DMEM with the same concentration (10^{-5} mol/L).

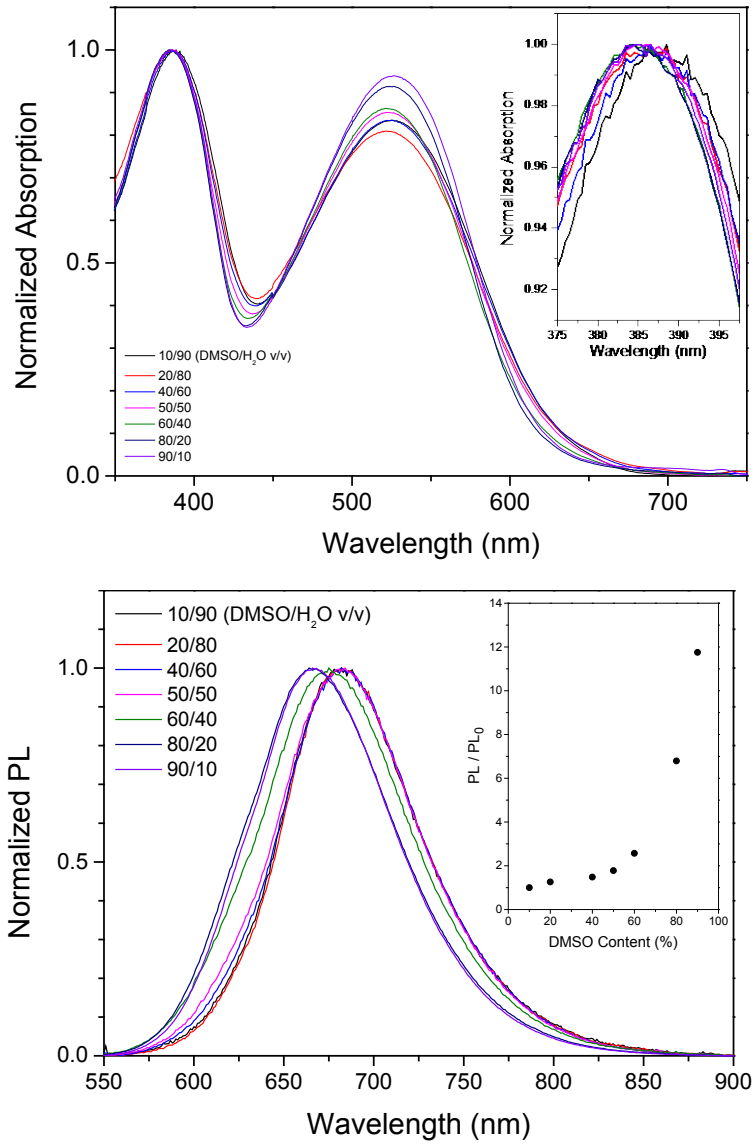


Figure S2. Absorption (upper) and emission (bottom) spectra of TbtPFN in DMSO/water solution.

With increase of the DMSO content in the mixed solvents, there exists a slight blue shift in the absorption spectra of TbtPFN. Simultaneously, the PL peaks show a significant blue shift, along with significantly enhanced emission intensity. The changes in the absorption and emission spectra are due to the amphiphilic structure of TbtPFN. In aqueous solution, the hydrophilic side chains stretch into water, while the hydrophobic backbones pack together. Thus the polymer adopts a structure in which the hydrophobic units are tucked inside and the hydrophilic units are exposed to water. The introduction of DMSO gives rise to the dissolution of the backbones, and then the "micro-phase separation" is disintegrated.[2]

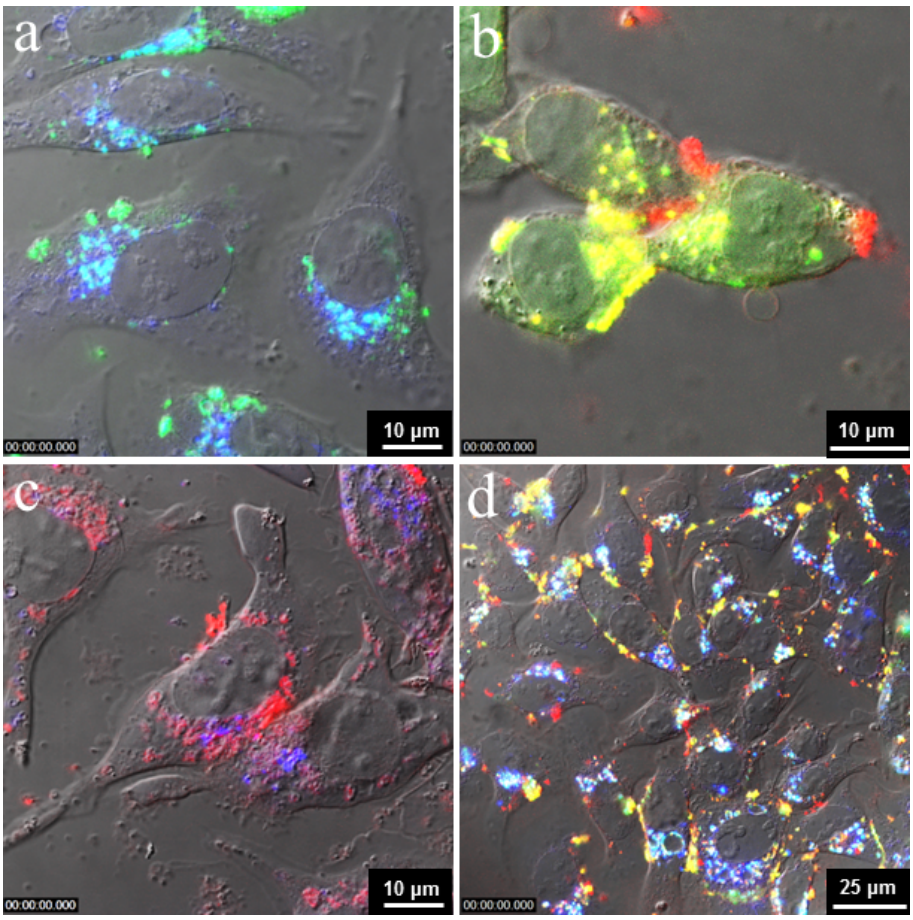


Figure S3. Cellular colocalization of multiple conjugated polymers. a: PFN/BtPFS dual-colocalization; b: BtPFS/TbtPFN dual-colocalization; c: PFN/TbtPFN dual-colocalization; d: PFN/BtPFS/TbtPFN tri-colocalization.

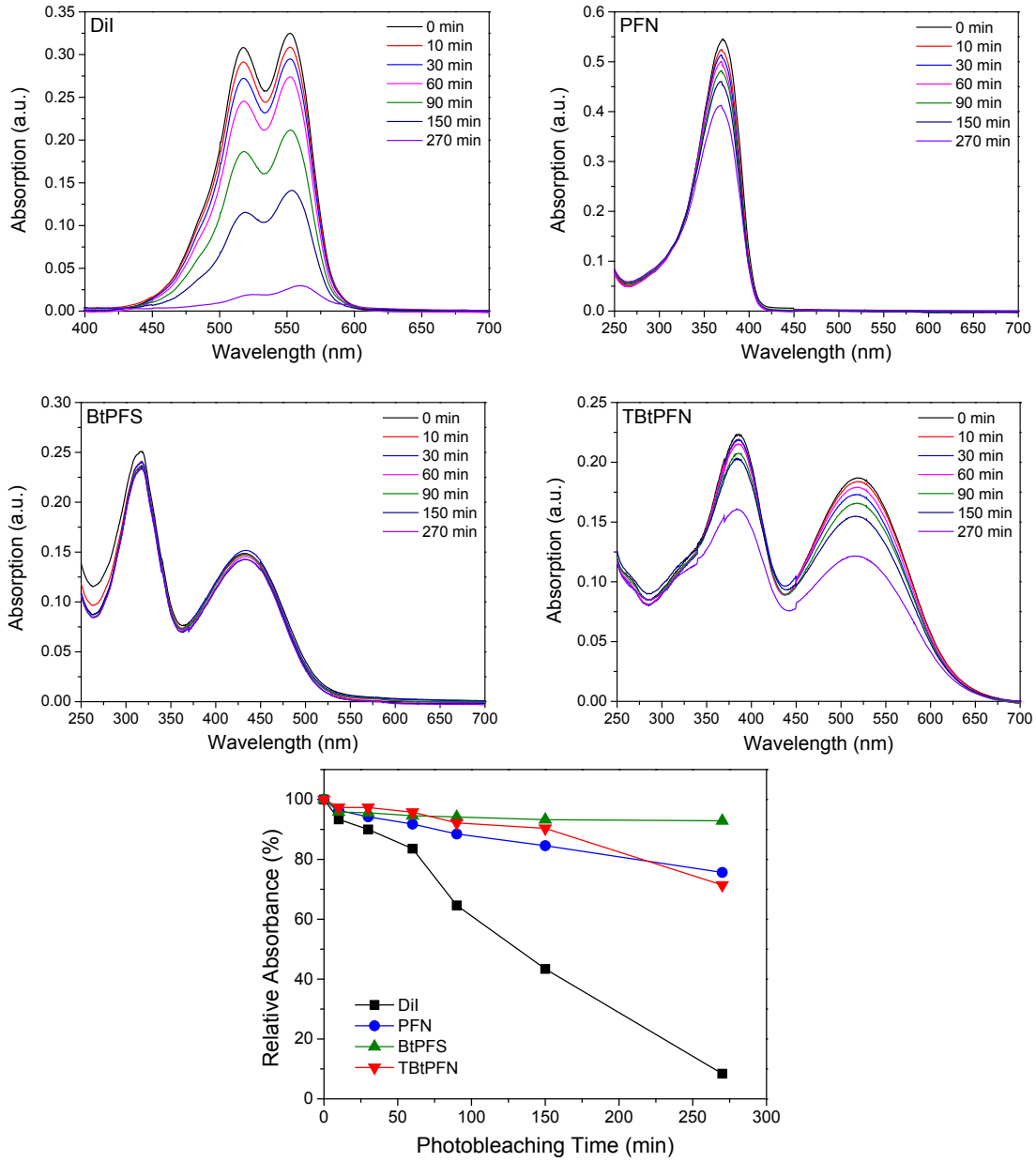


Figure S4. UV-vis spectra and maximum absorbance changes of DiI, PFN, BtPFS, and TbtPFN at different UV illumination time.

[1] i) F. Huang, H. Wu, D. Wang, W. Yang, Y. Cao *Chem. Mater.* **2004**, *16*, 708; ii) F. Huang, L. Hou, H. Shen, J. Jiang, F. Wang, H. Zhen, Y. Cao *J. Mater. Chem.* **2005**, *15*, 2499; iii) F. Huang, X. Wang, D. Wang, W. Yang, Y. Cao *Polymer* **2005**, *46*, 12010.

[2] i) T.-Q. Nguyen, I. B. Martini *Phys. Chem. B* **2000**, *104*, 237; ii) T. Nguyen, B. J. Schwartz *J. Chem. Phys.* **2002**, *116*, 8198.