Supporting Information

A Facile Strategy towards Conjugated Polyelectrolyte with Oligopeptide as Pendants for Biological Applications

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1. Materials

The toluene used for Suzuki polymerization was pretreated by sulfuric acid followed by distillation. NMR solvent, chloroform-D (99%) was provided by Cambridge Isotope Laboratories, Inc. MCF-7 breast cancer cells were provided by American Type Culture Collection. The peptide of propargyl-RRRRRRRRRR was purchased from GenicBio Limited company (Shanghai, China). All other chemicals and reagents were purchased from Aldrich or Merck and used as received. 2,7-Dibromo-9,9-bis(2-

(2-(2-bromoethoxy)ethoxy)ethyl)fluorene **1** was synthesized according to our previous report.[1]

2. Instrumentation

^{1}H and ^{13}C NMR spectra were reported on a Bruker Avance 500 NMR spectrometer in $CDCl_3$ using tetramethylsilane (TMS; δ = 0.00 ppm) as an internal reference. Fourier Transform Infrared (FTIR) spectra were recorded using a PE Paragon 1000 spectrometer (KBr disk). Gel permeation chromatography (GPC) analysis was conducted with Waters 996 photodiode detector and Phenogel GPC columns, using polystyrenes as the standard and THF as the eluent at a flow rate of 1.0 mL/min at 35 ^{o}C. UV-vis spectra were recorded on a Shimadzu UV-1700 spectrometer. Photoluminescence (PL) spectra were measured on a Perkin Elmer LS-55 equiped with a xenon lamp excitation source and a Hamamatsu (Japan) 928 PMT, using 90 degree angle detection for solution samples. All UV-vis and PL spectra were collected at 24 ± 1 ^{o}C. Fluorescence quantum yield was determined using quinine sulfate in 0.1 M H_2SO_4 aqueous (54%) as the standard. The absorbance of the solutions was kept below 0.1 to avoid internal filter effect. Fisher brand regenerated cellulose dialysis tubing with 12~14 kDa molecular weight cutoff was used for polymer dialysis. Laser light scattering (LLS) measurements were performed using Brookhaven instruments corporation (BIC) 90 plus with λ = 659 nm and angle = 90^{o}, and the particle diameters were calculated by ZetaPlus Particle Sizing Software Version 3.93. Atomic force microscopy (AFM) images were obtained by Dimension 3100 AFM (Veeco, CA, USA) under ambient conditions. Confocal laser scanning microscopy (CLSM) images

were recorded on a Zeiss LSM 410 (Jena Germany) CLSM with imaging software (Fluoview FV1000). MilliQ water (18.2 MQ) was used for all the experiments.

3. Synthesis

9,9-Bis(2-(2-(2-bromoethoxy)ethoxy)ethyl)-2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)fluorene (2). Anhydrous dioxane (10 mL) was added to a mixture of compound 1 (714 mg, 1 mmol), potassium acetate (700 mg, 7 mmol), bis(pinacolato)diboron (600 mg, 2.4 mmol) and $Pd(dppf)_2Cl_2$ (50 mg) in a Schlenk tube. The reaction mixture was stirred at 90 ^oC for 15 h, and then cooled to room temperature. After the solvent removal, the residual was redissolved in dichloromethane and washed with water. After drying over $MgSO_4$, the solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography (hexane/ethyl acetate = 5/1) to afford white crystals (541 mg, Yield: 67%). ^1H NMR (500 MHz, $CDCl_3$, δ): 7.89−7.70 (m, 4 H), 7.76−7.65 (m, 2 H), 3.66 (t, J = 6.4 Hz, 4 H), 3.38 (dd, J = 12.3 and 5.8 Hz, 8 H), 3.16 (t, J = 4.7 Hz, 4 H), 2.69 (t, J = 7.5 Hz, 4 H), 2.44 (t, J = 7.5 Hz, 4 H), 1.39 (s, 24 H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 148.51, 143.11, 134.05, 129.21, 119.52, 83.84, 71.08, 70.31, 69.89, 67.00, 51.04, 39.47, 30.11, 24.94.

Poly[9,9-bis(2-(2-(2-bromoethoxy)ethoxy)ethyl)fluorene] (P1). A Schlenk tube was charged with compound 1 (71.4 mg, 0.1 mmol), compound 2 (80.8 mg, 0.1 mmol), $Pd(PPh_3)_4$ (5 mg, 4 μmol) in toluene (7 mL) before it was sealed with a rubber septum. The Schlenk tube was degassed with three freeze-pump-thaw cycles to

remove air. After the mixture was heated to 85 ^{o}C, an aqueous Et_4NOH solution (20 wt %, 1.0 mL) was added to initiate the reaction. After 20 h, the reaction was stopped and cooled down to room temperature. The mixture was dropped slowly into methanol (100 mL) to precipitate the crude polymer. The crude polymer was collected by centrifugation, then was subsequently redissolved in dichloromethane (100 mL), washed with water 3 times, and dried over $MgSO_4$. After solvent concentration, P1 (77.5 mg, yield: 70%) was obtained as a white solid by precipitation in methanol. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.78−7.41 (m, 6 H), 3.67 (br, 4 H), 3.50−3.26 (br, 8 H), 3.25−3.12 (br, 4 H), 2.86−2.72 (br, 4 H), 2.52−2.40 (br, 4 H).

Poly[9,9-bis(2-(2-(2-azidoethoxy)ethoxy)ethyl)fluorene] (P2). To a mixture of P1 (30 mg) in THF/DMSO (20 mL/10 mL) was added NaN_3 (260 mg, 4 mmol). The reaction mixture was stirred at 70 ^{o}C for 2 days, and then cooled to room temperature. The mixture was diluted with dichloromethane to 150 mL and washed with water 5 times and dried over $MgSO_4$. After concentrating the solution to ~10 mL, P2 (24 mg, yield: 92%) was obtained as white solid by precipitation from methanol. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.84−7.51 (m, 6 H), 3.63 (br, 0.46 H), 3.54 (br, 3.54 H), 3.44 (br, 4 H), 3.29 (br, 8 H), 2.90−2.83 (br, 4 H), 2.59−2.47 (br, 4 H).

PF-R10. A Schlenk tube was charged with P2 (1 mg), $CuSO_4$ (1 mg), sodium ascorbate (2 mg) and THF (1 mL). After the P2 was completely dissolved, a peptide of propargyl-RRRRRRRRRR (10 mg) in DMSO (1 mL) solution was added slowly to the reaction mixture. The reaction was kept at room temperature under argon atmosphere for 4 days. After the reaction was completed, the product was purified by

dialysis against MilliQ water using 12~14 kDa molecular weight cutoff dialysis membrane for 3 days. The solution was further purified by running through a PD-10 column. The obtained PF-R10 was stored as aqueous solution in refrigerator at 4 ^{o}C.

4. Cell Culture

MCF-7 breast cancer cells were cultured in folate-free Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum and 1% penicillin streptomycin at 37 °C in a humidified environment containing 5% CO_2. Before experiments, the cells were precultured until confluence was reached.

5. Cellular imaging

MCF-7 breast cancer cells were cultured in chambers at 37 °C. After 80% confluence, the medium was removed and the adherent cells were washed twice with 1× PBS buffer. PF-R10 in DMEM medium at 5 μM was then added to chamber. After incubation overnight, the cells were washed three times with 1× PBS buffer and then fixed with 75% filtered ethanol for 10 min, which were further washed twice with 1× PBS buffer and imaged by confocal laser scanning microscope (CLSM, Zeiss LSM 410, Jena, Germany) with imaging software (Olympus Fluoview FV1000). The fluorescence signal from PF-R10 was collected from 430 to 470 nm upon 405 nm excitation with a laser power of 1.25 mW.

6. Cytotoxicity Assay by MTT Method

The cytotoxicity of PF-R10 against MCF-7 cancer cells was evaluated by MTT assay. Briefly, MCF-7 breast cancer cells were seeded in 96-well plates (Costar, IL, USA) at an intensity of 4×10^4 cells/mL. After 24 h incubation, the cells were exposed to a series of doses of PF-R10 at 37 °C. After the designated time intervals, the wells were washed twice with 1×PBS buffer and 100 μL of freshly prepared MTT (0.5 mg/mL) solution in culture medium was added into each well. The MTT medium solution was carefully removed after 3 h incubation in the incubator. Filtered DMSO (100 μL) was then added into each well and the plate was gently shaken for 10 min at room temperature to dissolve all the precipitates formed. The absorbance of MTT at 570 nm was monitored by the microplate reader (Genios Tecan). Cell viability was expressed as the ratio of the absorbance of the cells incubated with sample suspension to that of the cells incubated with culture medium only.

7. PL spectra of PF-R10 with NaOH concentration variation

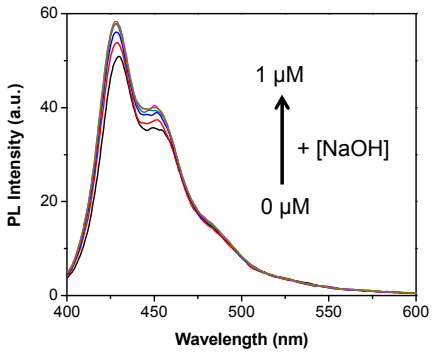

Figure S1. PL spectra of PF-R10 with [NaOH] ranging from 0 to 1 μM at interval of 0.2 μM. [PF-R10] = 4.5 μM based on repeat unit. Excitation at 380 nm.

8. Reference

(1) Pu, K. Y.; Fang, Z.; Liu, B. *Adv. Funct. Mater.* **2008**, *18*, 1321–1328.