Journal Name RSCPublishing

COMMUNICATION

# Supporting Information

## Cationic Fluorescent Polymer Core-Shell Nanoparticles for Encapsulation, Delivery, and Non-Invasively Tracking Intracellular Release of siRNA

*Ji-Cheng Yu, Sha Zhu, Pei-Jian Feng, Cheng-Gen Qian, Jun Huang, Min-Jie Sun, Qun-Dong Shen**

### Materials and methods

2,5-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophene and 2,7-dibromo-9,9-bis(3'-((N, N-dimethyl)-N-ethylammonium)propyl)fluorene were purchased from Hanhong Chemical Co. (Shanghai, China). Tetrakis-(triphenylphosphine) palladium (0) was purchased from Aldrich Chemical Co. Poly{9,9-di[3'-((N,N-dimethyl)-N-ethylammonium) propyl]-2,7-fluorenyl-*alt*-4,7-(2,1,3-benzothiadiazole) dibromide} (BtPFN) was synthesized according to the previous method.[s1]

Dulbecco's modified Eagles medium (DMEM, Gibco, USA) and bovine serum albumin (BSA) were purchased from Sunshine Biotechnology Co., Ltd (Nanjing). Anti-luciferase siRNA (sense sequence: 5'-CGU ACG CGG AAU ACU UCG AdTdT-3', anti-sense sequence: 5'-UCG AAG UAU UCC GCG UAC GdTdT-3') and negative control siRNA (sense sequence: 5'-UUCUCCGAACGUGUCACGUTT-3', anti-sense sequence: 5'-ACGUGACACGUUCGGAGAATT-3') were purchased from Shanghai GenePharma Co., Ltd. The siRNA with a Cy5 fluorophore on the 5' end of the sense strand was purchased from Ribobio Co., Ltd. (Guangzhou, China); Sense sequence: 5'- CGU ACG CGG AAU ACU UCG AdTdT-3', and anti-sense sequence: 5'- UCG AAG UAU UCC GCG UAC GdTdT-3'. Ethidium bromide was from Sangon Biotech Co., Ltd. (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) was purchase from the Beyotime Institute of Biotechnology (Shanghai). The Lipofectamine 2000 transfection kit was purchased from Invitrogen (Carlsbad, USA). The human hepatocellular liver carcinoma cell line HepG2 from the American Type Culture Collection (ATCC) were cultured in DMEM medium supplemented with 10% FBS (fetal bovine serum) and 1% penicillin/streptomycin in a sterile incubator at 5% $CO_2$ and 37$^{o}$C. The HepG2-luciferase cell line, which stably expresses luciferase, was obtained through transfection with a retrovirus carrying the luciferase gene according to a standard protocol and clones derived from discrete colonies were isolated and amplified in medium.

$^{1}$H-NMR spectrum was collected on a Bruker DRX-300 spectrometer. The UV-Vis absorption spectra were measured using a UV-Vis spectrophotometer (MAPADA UV-1800). Fluorescence spectra were recorded on a steady-state spectrofluorometer (HORIBA JOBIN YVON FM-4NIR). Particle size distribution and average hydrodynamic diameters were determined by a Brookheaven BI9000AT particle size analyzer. Zeta potentials were measured by a zeta potential analyzer (Malvern Zetasizer Nano-Z). Transmission electron microscopy (TEM) images were recorded on a JEOL JEM-2100 transmission electron microscope.

Synthesis of poly{9,9-di(3'-((N,N-dimethyl)-N-ethylammonium)propyl)-2,7-fluorenyl-*alt*-2,5-thienyl} (ThPFN)

2,5-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophene (0.24 g), 2,7-dibromo-9,9-bis (3'-((N,N-dimethyl)-N-ethylammonium)propyl)fluorene (0.51 g), and tetrakis-(triphenyl phosphine) palladium (0) (0.05 g) were dissolved in a degassed mixture of 40 mL of 0.3 mol/L $Na_2CO_3$ aqueous solution and 20 mL of N,N-dimethylformamide (DMF). The mixture was stirred at 90 $^oC$ for 48 h under argon atmosphere. After cooling down to room temperature, the mixture was poured into 500 ml of acetone. The precipitate was collected by centrifugation and then dissolved and dialyzed in distilled water using a membrane with a cutoff molecular weight of 8000 g/mol for 3 days. After vacuum drying at 60 $^oC$ for 24 h, 0.23 g of the final product was obtained (yield 45%). $^1H$ NMR (300MHz, $D_2O$): 8.46-7.23 (m, 8H), 3.04-2.88 (m, 8H), 2.62 (s, 12H), 2.29-2.22 (m, 4H), 1.05 (m, 10H).

Preparation of BtPFN nanoparticles

BtPFN (0.5 mg) was dissolved in DMSO (0.5 mL). Subsequently, this solution was added dropwise into deionized (DI) water (10 mL), and sonicated at room temperature using a microtip probe sonicator. The resulting solution was dialyzed in DI water using a membrane with a cutoff molecular weight of 8,000 g/mol for 24 h. BtPFN NPs suspension was filtered through a 0.45 μm microfilter, and then stored at 4°C.

Assembly of ThPFN/siRNA/BtPFN NPs

62.5 μL of siRNA (20 nmol/L) was added into 1.8 mL BtPFN NPs suspension, and incubated at room temperature for 15 minutes. The free siRNA was removed by thorough ultrafiltration (MWCO: 100,000 g/mol). Subsequently, 31.25 μL ThPFN ($10^{-3}$ mol/L) was added into the obtained siRNA/BtPFN NPs suspension and incubated for another 15 minutes. The free ThPFN was removed using the same method. The electrostatic charge ratio of ThPFN/siRNA/BtPFN is 1:1:5.

Agarose gel electrophoresis

Samples (free siRNA, siRNA/BtPFN, and ThPFN/siRNA/BtPFN) were incubated with 10% fetal bovine serum (FBS) at 37$^oC$ for 4 h. Then, 1 mmol/L sodium dodecyl sulfate solution were added into each sample and the mixtures were further incubate for 30 min. The samples were loaded on a 1.5% agarose gel containing 0.5 μg/mL ethidium bromide and the gel electrophoresis was carried out at 100V for 20 min. The gel was subsequently imaged using a UV imaging system (Clinx Science Instruments 1560).

Cellular uptake of ThPFN/siRNA/BtPFN NPs and fluorescence imaging

Cells were seeded in confocal microscope dishes at the density of $1\times10^5$/mL in complete DMEM and cultured for 24 h at 37 °C in a humidified atmosphere containing 5% $CO_2$. Then, the medium was replaced by DMEM which has no FBS and contains ThPFN/siRNA/BtPFN NPs where the final siRNA concentration is 50 nmol/L. After incubation for 4 h, the cells were washed twice with PBS (pH 7.4). Cellular uptake of ThPFN/siRNA/BtPFN NPs was determined by a confocal laser scanning microscope (Zeiss LSM-710) at excitation wavelength of 405 nm.

Transfection of siRNA and silence of luciferase

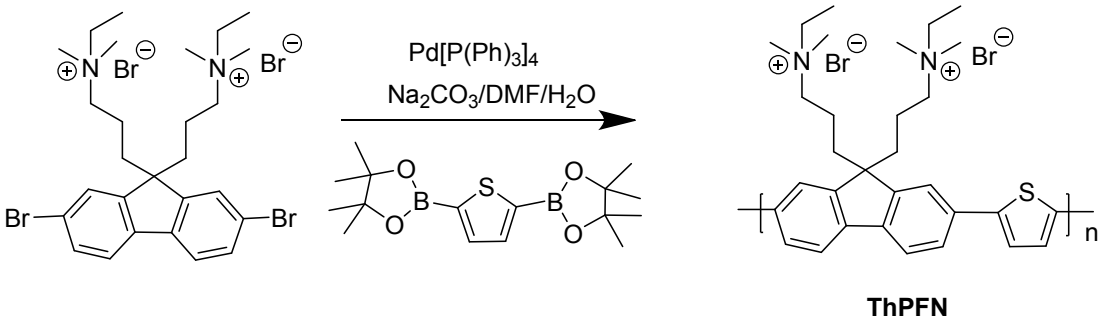
HepG2 cells stably transfected with luciferase reporter gene were seeded in 12-well plates at the density of $1\times10^4$ cells per well in 1.5 mL DMEM with 10% FBS but no penicillin/streptomycin and cultured at 37 $^oC$ in a humidified atmosphere containing 5% $CO_2$ for 24 h. The complete medium was replaced by DMEM containing ThPFN/siRNA/BtPFN NPs with the final siRNA concentration of 50 nmol/L. siRNA/BtPFN NPs, commercially available Lipofectamine 2000 containing the same amount of siRNA, and naked siRNA were also transfected as control groups. Medium containing luciferase silent siRNA

was cultured with HepG2 cells at 37 °C for 5 h. Then the culture medium was replaced with fresh complete DMEM with both 10% FBS and 1% penicillin/streptomycin and incubated for 24 h. After that, cells were washed and lysed. The rest of luciferase in cells left in supernatant was measured according to the intensity of bioluminescence during luciferin oxidization process, which is oxidized under the catalytic effects of luciferase, on GloMax-Multi Jr Single Tube Multimode Reader (Promega) with a Luminescence Module, using a luciferase assay kit (Beyotime, China) on the basis of the standard protocol provided by the supplier. Meanwhile the relative light units (RLU) were normalized against protein concentrations in the cell extracts, which were measured according to a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL).

Cytotoxicity analysis

MTT assay was exploited to determine the cytotoxicity of different materials (BtPFN, ThPFN, BtPFN/siRNA/ThPFN NPs). HepG2 cells were seeded in a 96-well plate at the density of $0.5 \times 10^4$ cells per well in DMEM containing 10% FBS and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere containing 5% $CO_2$ for 24 h. Then the medium was replaced by DMEM supplemented with indicated doses of different materials and cultured for 5 h. The final siRNA concentrations in the culture medium were 50 nmol/L. Then the treated cells were incubated in completed medium for an additional 20 h. 10 μL of MTT (0.5 mg/mL) solution was added to each well and incubated for 3 h. Then, the supernatant was removed and the products were lysed with 200 μL of DMSO. The absorbance values were recorded at 580 nm using a microplate reader (Thermo Electron Corporation). The absorbance of the untreated cells was used as a control and its absorbance was set as the reference value for calculating 100% cellular viability.




**ThPFN**

**Scheme S1**. Schematic preparation and molecular structure of the conjugated polymer ThPFN.

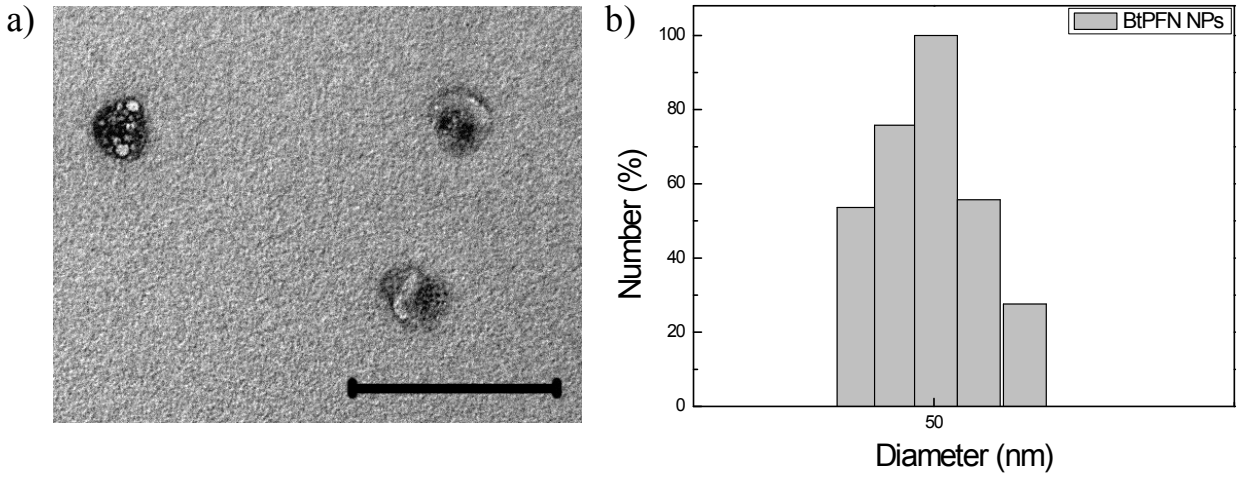



**Figure S1.** a) TEM image and b) hydrodynamic diameter distribution of BtPFN NPs. (Scale bar: 200 nm)

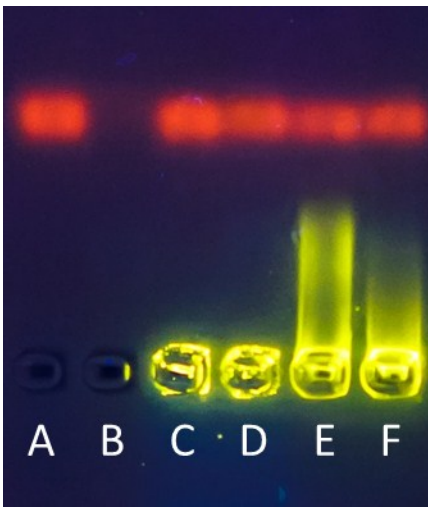



**Figure S2.** Agarose gel electrophoresis: Lane A) free siRNA, B) free siRNA incubated with 10% FBS, C) ThPFN/siRNA/BtPFN, D) ThPFN/siRNA/BtPFN incubated with 10% FBS, E) siRNA/BtPFN, F) siRNA/BtPFN incubated with 10% FBS. All NPs were disassembled using a sodium dodecyl sulfate solution before electrophoresis. siRNA bands (Red) were stained with ethidium bromide.

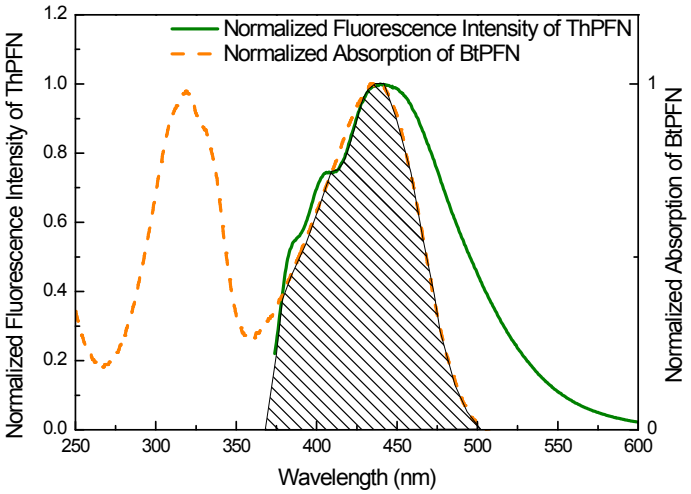



**Figure S3.** Normalized fluorescence emission spectrum of ThPFN (dash line) and electronic absorption spectrum of BtPFN (solid line).

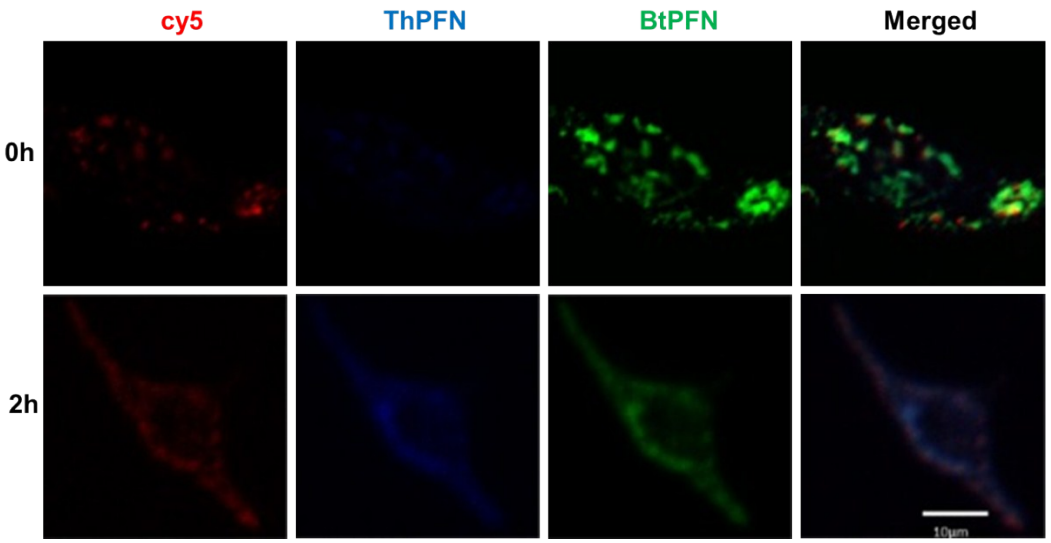



**Figure S4.** CLSM images of HepG2 cells incubated with ThPFN/Cy5-siRNA/BtPFN NPs after 0h (upper row) and 2h (bottom row) transfection. The red channel was Cy5; the blue channel was ThPFN; the green channel was BtPFN.

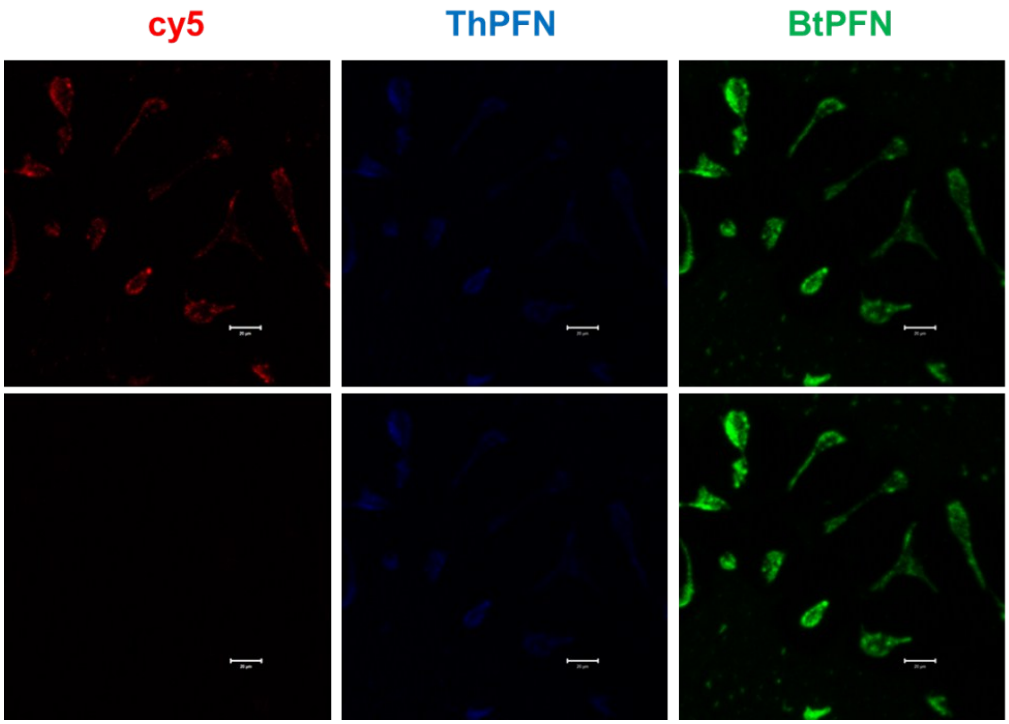



**Figure S5.** CLSM images of HepG2 cells incubated with ThPFN/Cy5-siRNA/BtPFN NPs pre- (upper row) or pro-exposure (bottom row) to the laser light. The red channel was Cy5; the blue channel was ThPFN; the green channel was BtPFN.

**Table S1.** Mean hydrodynamic diameters and zeta potentials.

| | Diameter (nm) | Zeta potential (mV) |
|---|---|---|
| BtPFN NPs | 50.2 | +37 |
| siRNA/BtPFN NPs | 64.2 | -6 |
| ThPFN/siRNA/BtPFN NPs | 82.8 | +23 |

## References

[s1] B. Sun, M. J. Sun, Z. Gu, Q. D. Shen, S. J. Jiang, Y. Xu, Y. Wang, *Macromolecules* 2010, **43**, 10348.