Bright Red Fluorescent Conjugated Polymer Nanoparticles with Dibenzopyran as Electron Donor for Cell Imaging

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Reagent and equipment:

Dulbecco's modified Eagle's medium (DMEM), Plutonic-F127 and poly(styrene-co-maleic anhydride) were purchased from Sigma-Aldrich. Other media components were obtained from Sigma-Aldrich too. Fetal bovine serum (FBS) was obtained from Hyclone laboratories Inc. 4-aminobutryrate hydrochloride, glycine, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Aladdin Industrial Inc. Millipore Simplicity 185 purification unit purified water (18.2 MΩ cm) used for rinsing and preparing all aqueous solutions. All Other reagents were obtained commercially and used without further purification. Absorbance spectra were

recorded on Purkinje General TU-1950 UV-Vis Spectrophotometer. Fluorescence emission and excitation spectra were recorded using a Perkin-Elmer LS-55 Spectrofluorometer. Quantum yields were measured by using a HAMAMATSU C9920-02G absolute PL quantum yield spectrometer. Fluorescence microscopy images were recorded on Olympus FV-1200 laser confocal microscope. Conjugated polymer PDBPTBT-2 was synthesized according to the previous report. [1] Number- (M_n) and weight-average (M_w) molecular weights were measured by gel permeation chromatography (GPC) on a Waters GPC2410 with THF as an eluent calibrated with polystyrene standards. TEM images were obtained on the JOEL JEM-2100 HR-TEM. Dynamic light scattering (DLS) were measured on Brookhaven BI-90Plus laser particle size analyzer. Zeta potential was measured on Beckman Coulter Delsa Nano C zeta potential measurement analyzer.

Preparation of CPNs:

1) For PDBPTBT-2/Pluronic F-127 nanoparticles: firstly, pluronic F-127 was dissolved in THF to make a 0.1 g/L solution. The conjugated polymers were also dissolved in THF to make a 0.05 g/L solution, which was mixed with the Pluronic F-127 solution. Secondly, a 2 mL quantity of the mixture solution was added rapidly to 8 mL of deionized water while sonicating the mixture. Finally, the excess THF can be removed on a rotary evaporator at 40℃ keeping concentration at 0.005 g/L.

2) For PDBPTBT-2/PMSA nanoparticles: Firstly, PSMS was dissolved in THF to make a 0.1 g/L solution. The conjugated polymers were also dissolved in THF to make a 0.05 g/L solution, which was mixed with PSMA solution. Secondly, a 2 mL

quantity of the mixture solution was added rapidly to 8 mL of deionized water while sonicating the mixture. Finally, the excess THF can be removed on a rotary evaporator at 40℃ keeping concentration at 0.005 g/L.

3) For PDBPTBT-2/PMSA nanoparticles with surface partially modified esters: a) high concentration PDBPTBT-2/PMSA nanoparticles were prepared first for modification. PDBPTBT-2 and PSMA were first dissolved in THF to make a stock solution. The two polymer solutions were diluted and mixed in THF to produce a solution mixture with a PDBPTBT-2 concentration of 0.05 g/L and a PSMA concentration of 0.02 g/L by sonicating to form a homogeneous solution. A 7 mL quantity of the solution mixture was quickly added to 14 mL of Milli-Q water in a vigorous bath sonicator. The excess THF was removed by a rotary evaporator at 40℃ keeping concentration at 0.05 g/L; b) In a typical conjugation reaction, 60 μl of 1 M pH 7.3 HEPES buffer were added to 3 mL of high concentration PDBPTBT-2/PMSA nanoparticles (0.05 g/L) from step a), resulting in a CNPs solution in 20 mM HEPES buffer with a pH of 7.3. Then, 30 μL, 15 μL, 3 μL, 0 μL of Ethyl 4-aminobutyrate hydrochloride at 1 mg/mL was added to the solution, respectively. 60 μL of freshly prepared EDC solution (5 mg/mL) and 0 μL, 20 μL, 36 μL, 40 μL of glycine at 0.45 mg/mL were added to the solution to generate CNPs with different surface modification rate. Last the above mixtures were magnetically stirred for 4 hours at room temperature. Finally, the resulting CNPs were dialyzed against water for at least 2-3 days in dark. The concentration of resulting CNPs solutions was calibrated according to UV-vis absorbance.

Cell culture:

HCT-116 cells were obtained from Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Cells were maintained in DMEM and supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine in a 5% CO_2 humidified incubator at 37°C. Cells were passed with a fixed ratio of 1:3 in every two days.

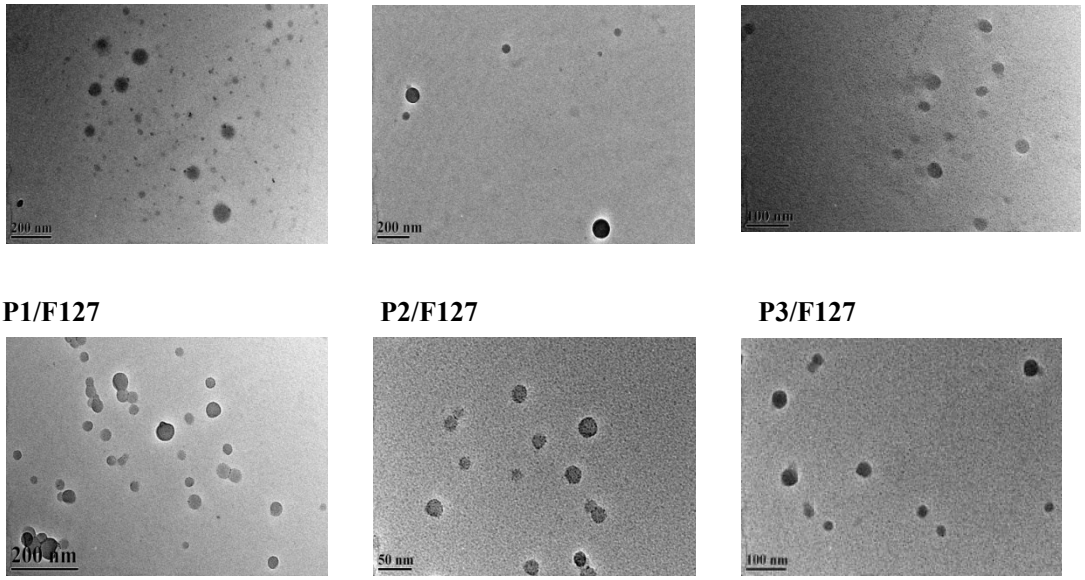

Figure S1 TEM images of CPNs.

Table S1. Molecular weight of PDBPTBT CPs

PDBPTBT	Molecular weight
P1	**866**
P2	**7595**
P3	**33313**

Table S2. Quantum yield of PDBPTBT CPs in polystyrene (PS) solid film[a]

PDBPTBT	Quantum yield (φ, %)
P1	40.0
P2	28.8
P3	10.3

[a] solid thin film was prepared at mass ratio of PS : CPs = 1:1.

1. a) J. Song, Y. Guo, L. Liu, H. Wang, *Dyes and Pigments* **2015**, *122*, 184-191; b) Y. Zhou, M. Li, Y. Guo, H. Lu, J. Song, Z. Bo, H. Wang, *ACS Appl. Mater. Interfaces* **2016**, *8*, 31348-31358.