Supporting Information:

Size-tunable and Crystalline BODIPY Nanorods for Bioimaging

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1. Experimental Procedures

Materials. BDP and its derivatives were prepared following the protocol has been reported. [S1] Milli-Q water was collected from a Milli-Q system (Millipore, USA). Chemicals and reagents were acquired from commercial sources without further purification.

The instruments for characterizations. The size distribution was performed using a Zeta-sizer Nano-ZS (Malvern Instruments Ltd.). Transmission electron microscopy (TEM) images were taken by a JEOL JEM-1011 (Japan) at the accelerating voltage of 100 kV. UV−vis absorption spectra were recorded via a Shimadzu UV-2450 UV−vis scanning spectrophotometer. Fluorescence emission spectra were conducted on a LS-55 fluorophotometer. The cell confocal images were obtained using Zeiss confocal laser microscope (ZEISS LSM 700). PXRD was performed by a Riguku D/MAX2550 diffractometer using CuKα radiation, 40 kV, 200 mA with scanning rate of 0.4 ^{o} min^{-1}. High-resolution TEM (HRTEM) images were recorded with a FEI-TECNAI G2 transmission electron microscope operating at 200 kV. Fluorescence quantum efficiency was obtained on a Hamamatsu Absolute PL Quantum Yield Measurement System C9920-02.

Preparation of BDP-NRs. BDP-NRs were prepared using a reprecipitation method. In a typical procedure, the BDP solution (400 μL) was quickly dropwise dispersed into 4 mL of milli-Q water with vigorous stirring at room temperature for 30 min. Then the solution was dialyzed against Milli-Q water for 24 h, the cutoff molecular weight of the dialysis bags is 3500.

Monitoring of the self-assembly of BDP. the BDP solution (400 μL) was quickly dropwise dispersed into 4 mL of milli-Q water with vigorous stirring at room temperature. Then we taked out 10 μL mixing solution at different time points (2.5, 5, 10, 15, 20 and 25 min), and diluted the solution using 90 μL of milli-Q water to maintain the status of the particels in mixing solution. Then we used TEM to observe the results.

Size tunability of BDP-NRs. Different volume of the BDP solution from 200 μL to 800 μL were quickly dropwise dispersed into 4 mL of milli-Q water with vigorous stirring at room temperature for 30 min, respectively. Then the solutions were dialyzed against Milli-Q water for 24 h, the cutoff molecular weight of the dialysis bags is 3500.

Measurement of fluorescence quantum yield. We firstly detected the concentration of BDP in BDP-NRs by absorbance curve. Then we prepared BDP acetone solution with the same concentration. Fluorescence quantum efficiency was obtained on a Hamamatsu Absolute PL Quantum Yield Measurement System C9920-02.

Cell culture. HeLa cells were propagated to confluence in DMEM medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin and 10% FBS, and maintained at 37^{o}C in a humidified atmosphere of 5% CO_2 for further cell experiments.

Biocompatibility of BDP and BDP-NRs in vitro by MTT Assay. Cells harvested in a logarithmic growth phase were seeded in 96-well plates at a density of 8×10^3 cells per well and incubated in DMEM for 24 h. The medium was then replaced by 200 μL

of DMEM containing predetermined concentrations of BDP and BDP-NRs, respectively, and then incubated for 24 h, followed by MTT assays to measure the live cells. Cell viabilities were determined by reading the absorbance of the plates at 490 nm with a microplate reader. The cells incubated with DMEM were used as the control. The cell viability (%) =A sample /A control ×100%. We also detected the cytotoxicity of BDP-NRs with HeLa cells for different culture time and concentration.

Cellular uptake and tracking in vitro. The cellular uptake measurement was investigated by CLSM. Cells harvested in a logarithmic growth phase were seeded in 6-well plates at a density of 2.5×10^5 cells/well and incubated in DMEM for 24 h. The medium was then replaced by 2 mL of DMEM containing BDP or BDP-NRs and incubated for different hours at 37°C, and further washed using PBS for 3 times. For the CLSM, the cells were fixed with 4% of paraformaldehyde solution for 10 min. After that, DAPI was added for another 5 min incubation to locate the nucleus. Later, the cells were washed with PBS and observed using confocal laser scanning microscopy (CLSM, Zeiss LSM 700).

2. Figures and Tables

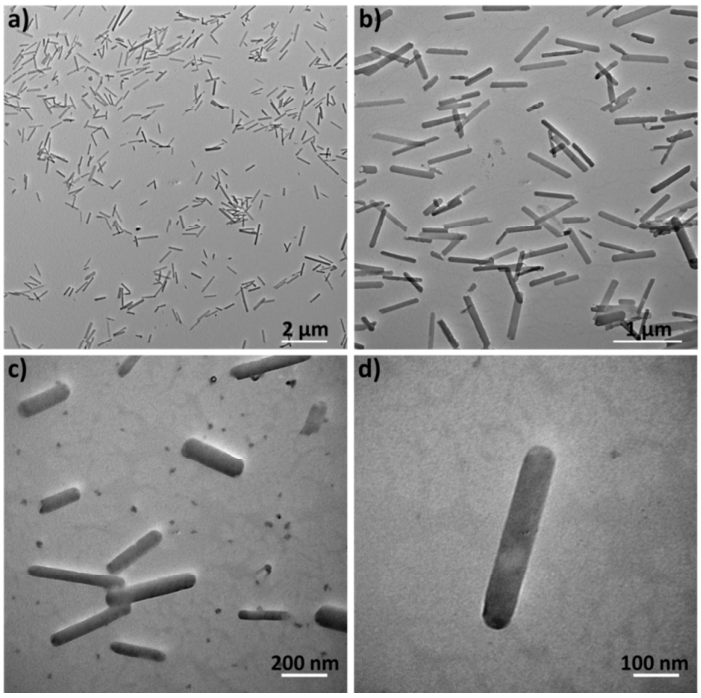

Figure S1. TEM pictures of BDP-NRs under different magnification. a) 10000 X; b) 30000 X; c) 100000 X; d) 200000 X.

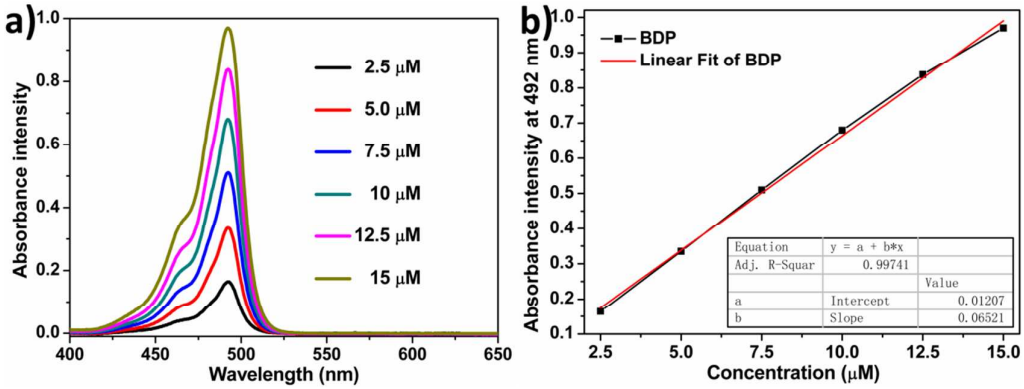

Figure S2. a) and b) Standard absorbance curve of BDP. (The absorbance of BDP molecules at 492 nm (from a mixture of acetone and water (v/v = 4:1)) as a function of BDP concentration.)

Table S1. Quantum yield and fluorescence lifetime of BDP and BDP-NRs, respectively.

	Quantum yield (ø)	Fluorescence lifetime (ns)
Free BDP in acetone	85.3%	6.28
BDP-NRs in water	5.5%	3.77

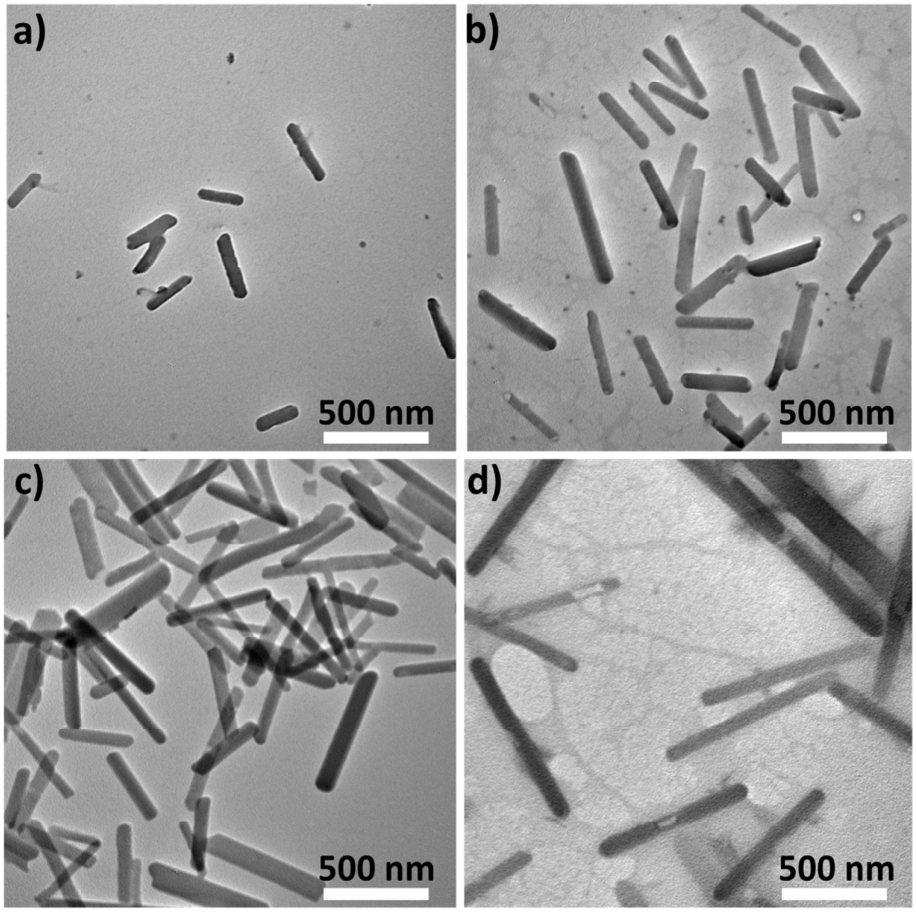

Figure S3. TEM pictures of BDP-NRs with different sizes. a) ~260 nm; b) ~420 nm; c) ~560 nm; d) ~920 nm. Scale bars: 500 nm.

Table S2. The relevant parameters of BDP-NRs made by adjusting the volume of BDP.

Volume of BDP (500 μM in acetone) (μL)	Length of BDP-NRs (nm)	Diameter of BDP-NRs (nm)	Aspect ratio
200	267.4±34.7	58.7±7.2	~4:1
400	423.8±40.5	61.3±4.3	~7:1
600	566.5±28.6	56.2±9.1	~9.5:1
800	925.1±51.4	63.9±6.6	~15:1

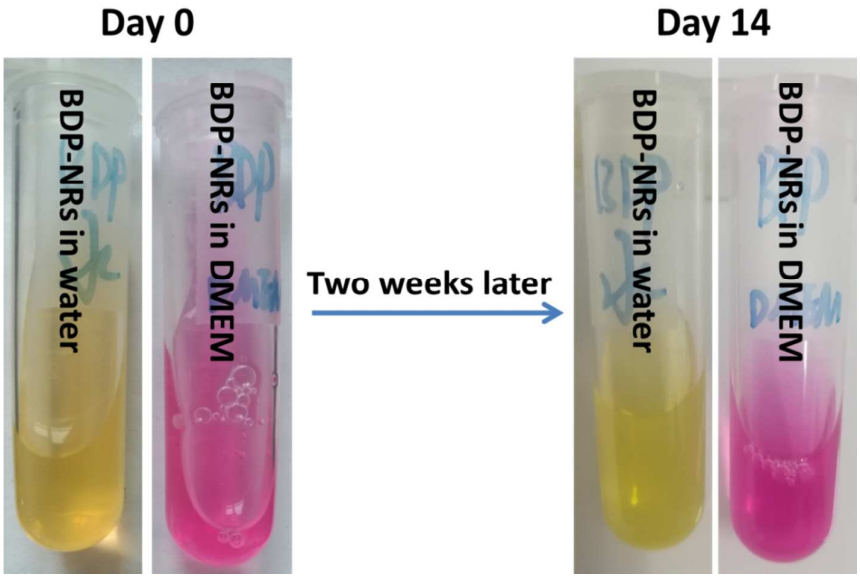

Figure S4. Photographs of BDP-NRs in different conditions which were freshly made and two weeks after preparation, respectively.

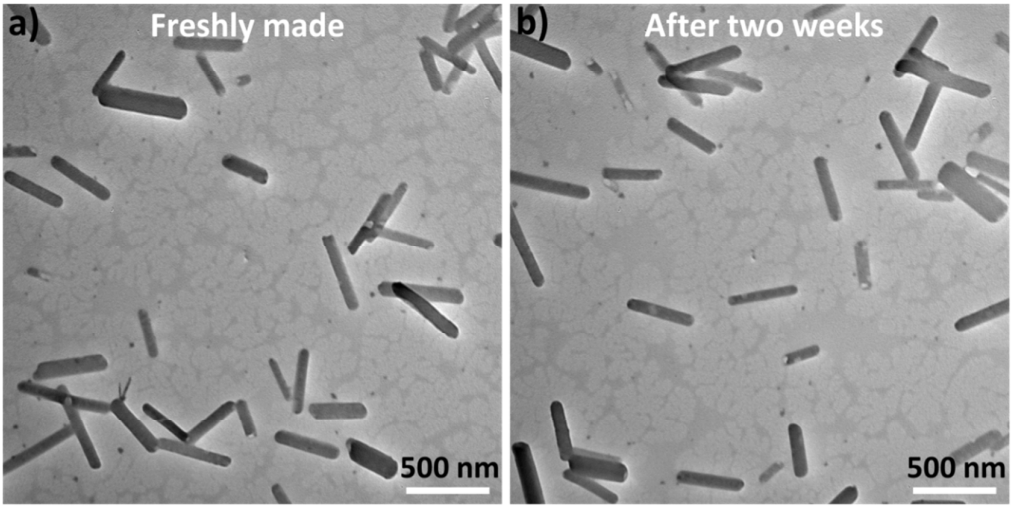

Figure S5. TEM images of BDP-NRs which were freshly made and two weeks after preparation. Scare bar: 500 nm.

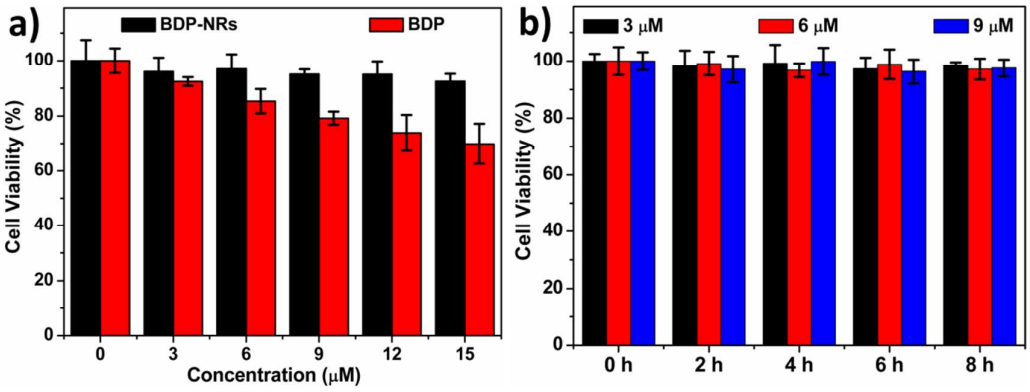

Figure S6. a) Relative cell viabilities of HeLa cells incubated with different concentrations of BDP-NRs and BDP for 24 h, respectively. b) Relative cell viabilities of HeLa cells incubated with different concentrations of BDP-NRs for different hours, respectively.

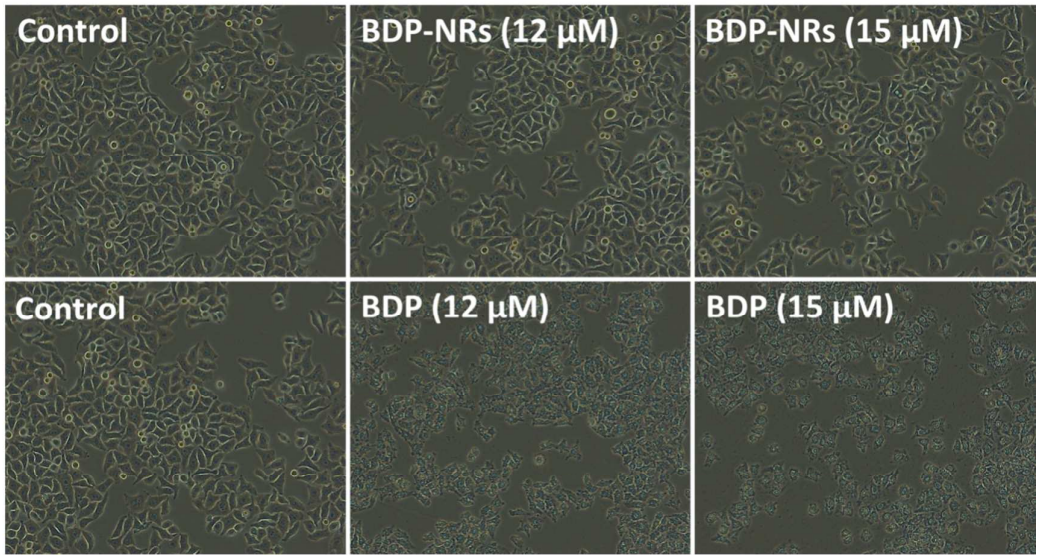

Figure S7. The morphology change of HeLa cells incubated with BDP or BDP-NRs of different concentration of BDP for 24 h at 37 ^{o}C.

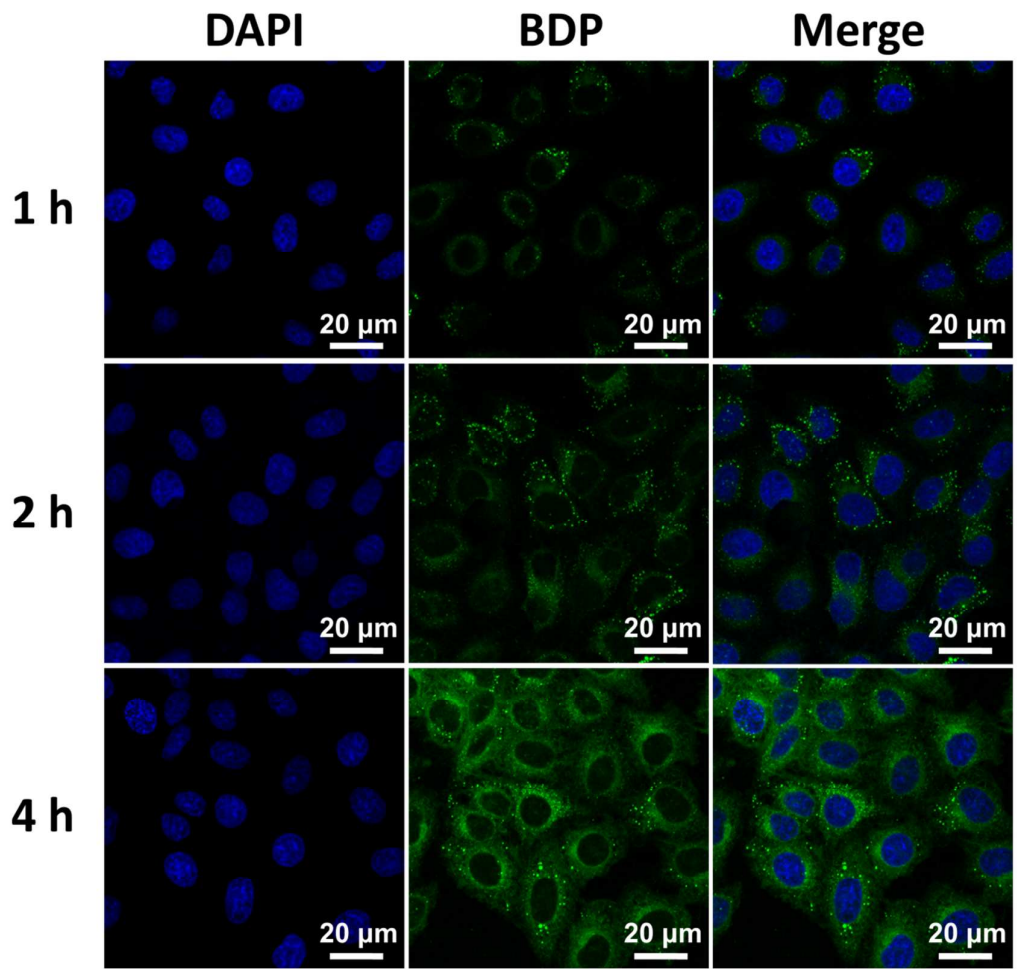

Figure S8. CLSM images showing changes in the signal of BDP in HeLa cells treated with BDP-NRs with the same BDP concentration of 9 μM for 1 h (upper), 2 h (middle) and 4 h (lower) at 37 ^{o}C. Scale bars: 20 μm.

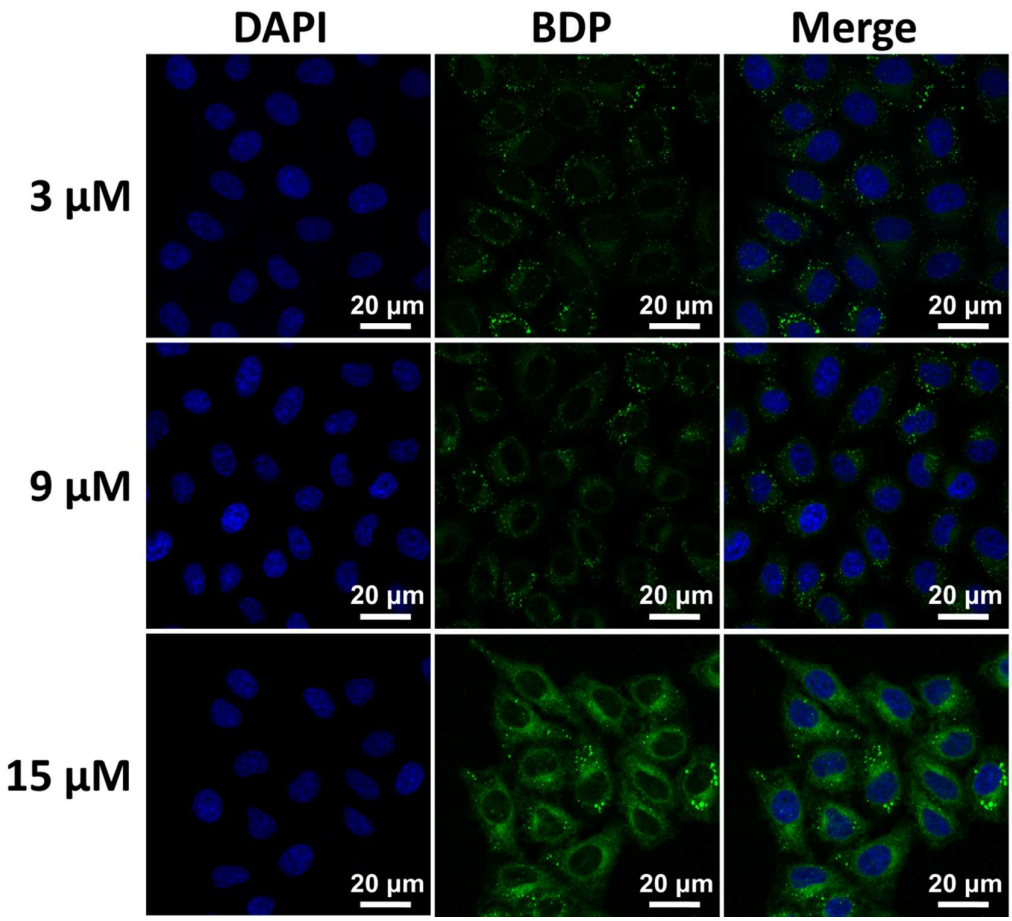

Figure S9. a) CLSM images showing changes in the signal of BDP in HeLa cells treated with BDP-NRs with the BDP concentration of 3 μM (upper), 9 μM (middle), 15 μM (lower) for 2 h at 37 ^{o}C. Scale bars: 20 μm.

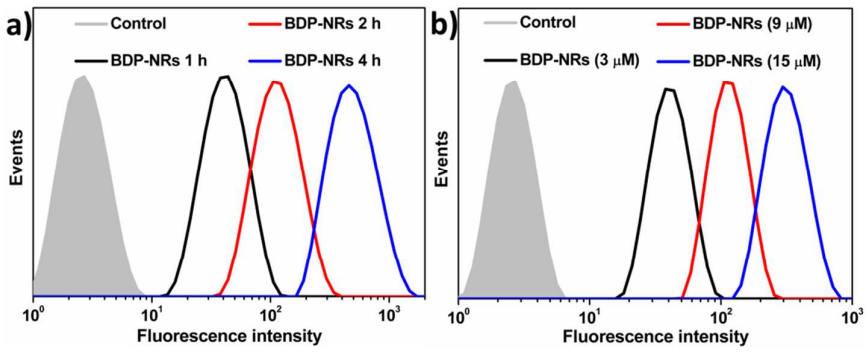

Figure S10. a) Flow cytometry analysss of the cellular uptake of BDP-NRs with the same concentration of BDP (9 μM) over different time (1, 2 and 4 h) at 37 °C. b) Flow cytometry analysis of the cellular uptake of BDP-NRs with various concentration of BDP (3, 9 and 15 μM) over 2 h at 37 °C.

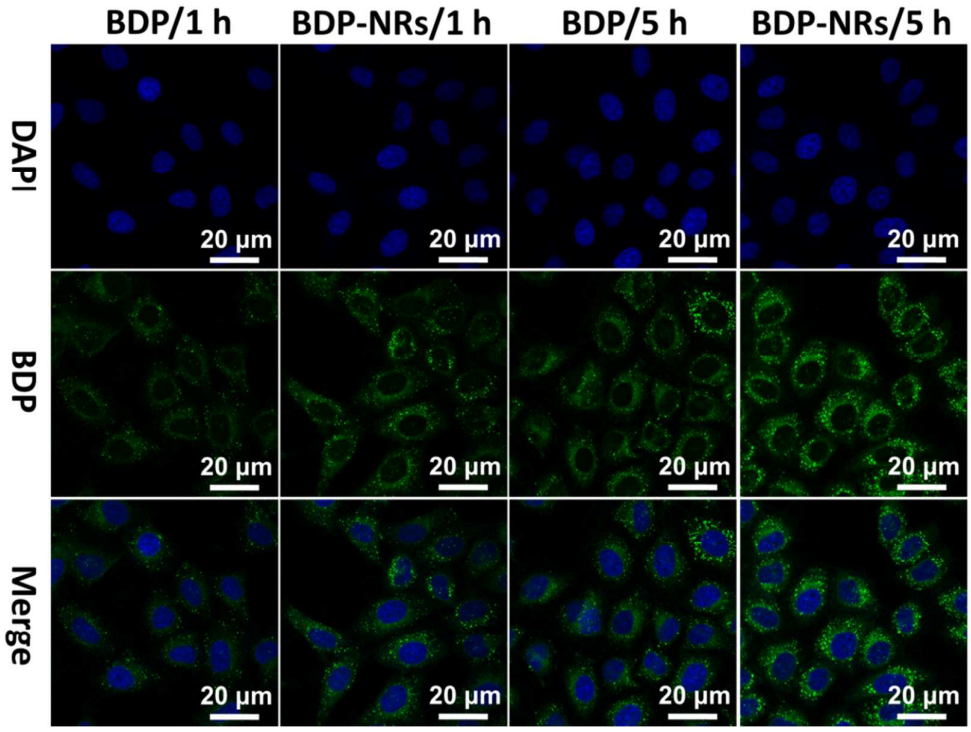

Figure S11. CLSM images showing changes in the signal of BDP or BDP-NRs in HeLa cells treated with the same BDP concentration of 9 μM for 1 h and 5 h at 37 ^{o}C. respectively. Scale bars: 20 μm.

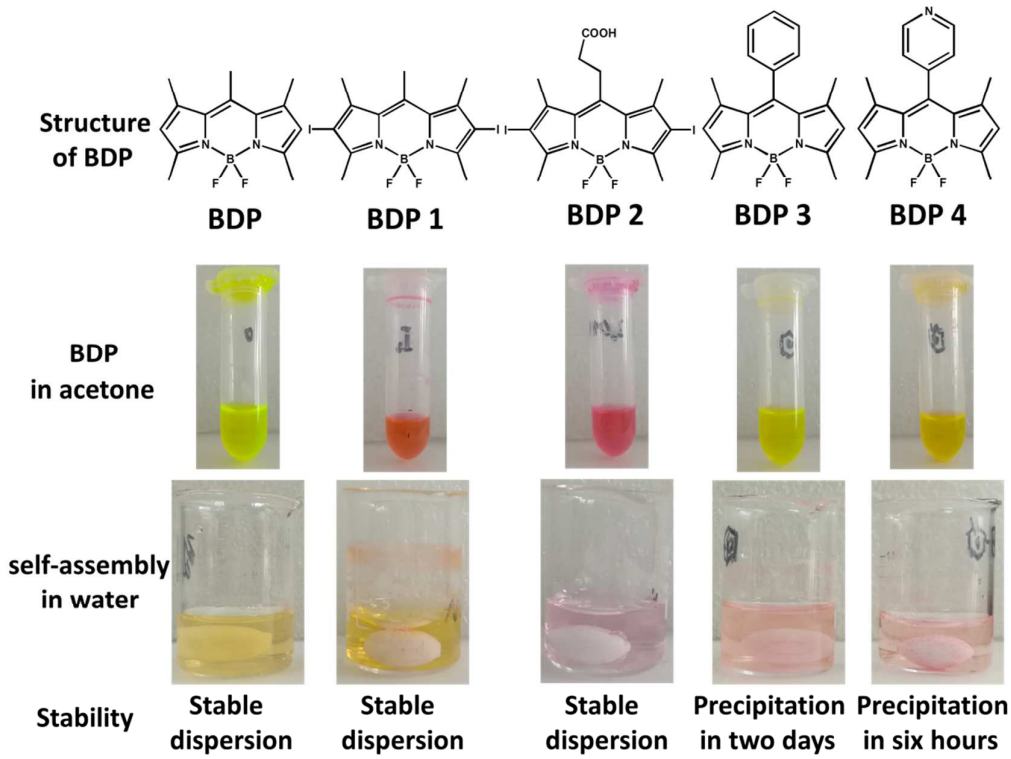

Figure S12. Structures of BDP and its derivatives (upper pictures)**.** Photographs of BDP and its derivatives in acetone (middle pictures). Photographs of BDP and its derivatives self-assembly in water (lower pictures), respectively.

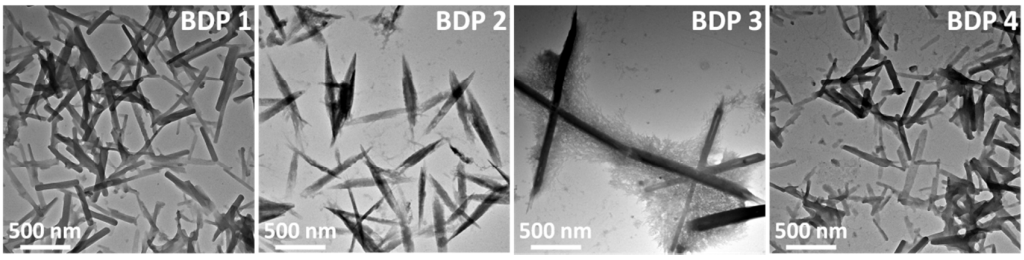

Figure S13. The TEM images of BDP derivatives self-assembly in water.

3. References

[1] A. Loudet, K. Burgess, *Chem. Rev.* **2007**, *107*, 4891-4932.