

# A Boron Difluoride Dye with Aggregation-Induced Emission Feature and Highly Sensitive to Intra- and Extra-Cellular pH Changes

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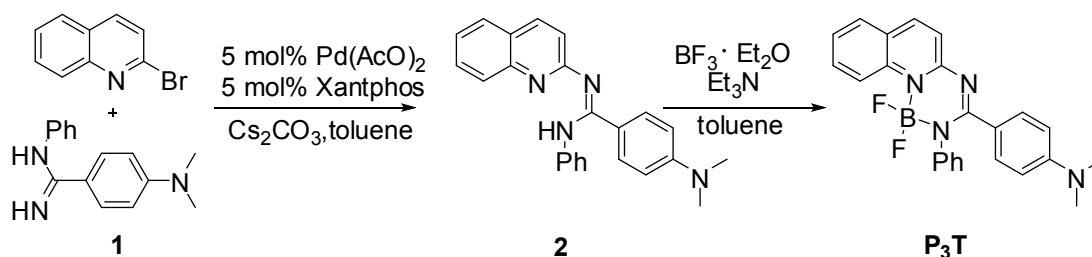
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### *1. Materials and methods*

Solvents were either used as purchased or dried according to procedures described in the literature. Compounds **1**,<sup>S1</sup> **2** and **P<sub>3</sub>T** were prepared according to the literature procedures. NMR spectra were detected with a Bruker Avance DMX 400 spectrophotometer or a Bruker Avance DMX 500 spectrophotometer with use of the deuterated solvent as the lock and the residual solvent or TMS as the internal reference. Low-resolution electrospray ionization mass spectra were recorded with a Bruker Esquire 3000 Plus spectrometer. High-resolution mass spectrometry experiments were performed with a Bruker Daltonics Apex III spectrometer. Transmission electron microscopy investigations were carried out on a HT-7700 instrument. UV-vis spectra were taken on a Shimadzu UV-2550 UV-vis spectrophotometer. The fluorescence experiments were conducted on a RF-5301 spectrofluorophotometer (Shimadzu Corporation, Japan). The single-crystal structures were analyzed on a Gemini A Ultra single crystal diffractometer. The cell images were taken with a confocal laser scanning microscopy (CLSM, Radiance2100, Bio-Rad) with a 100 × oil immersion lens. Density functional theory (DFT) calculations were performed with the Gaussian 09 program. Geometry optimizations and frequency calculations were performed with B3LYP functional using 6-31+G(d) basis set for all the atoms in gas phase.

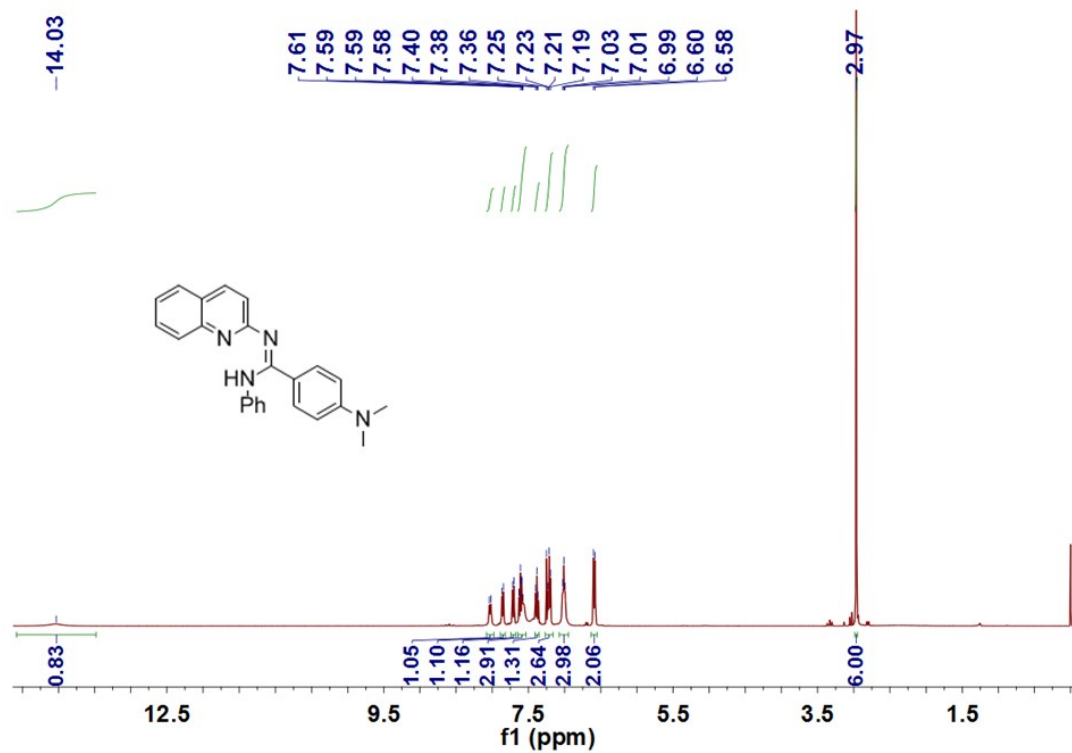
## 2. Synthesis of **P<sub>3</sub>T**



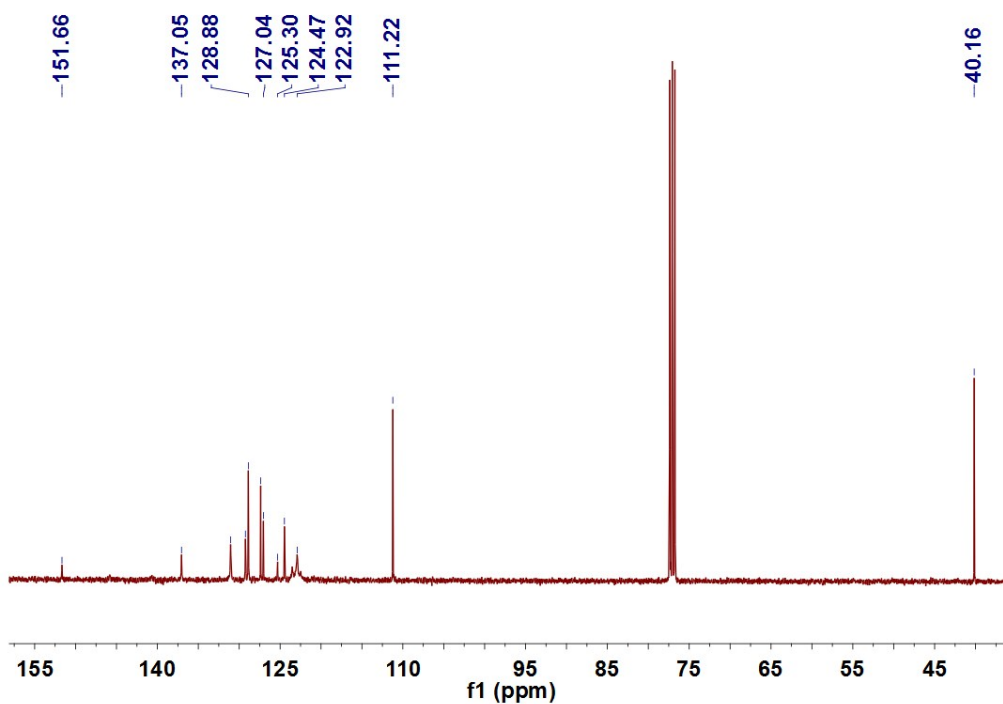
**Scheme S1.** Synthesis of **P<sub>3</sub>T**.

### 2.1 Synthesis of compound **2**

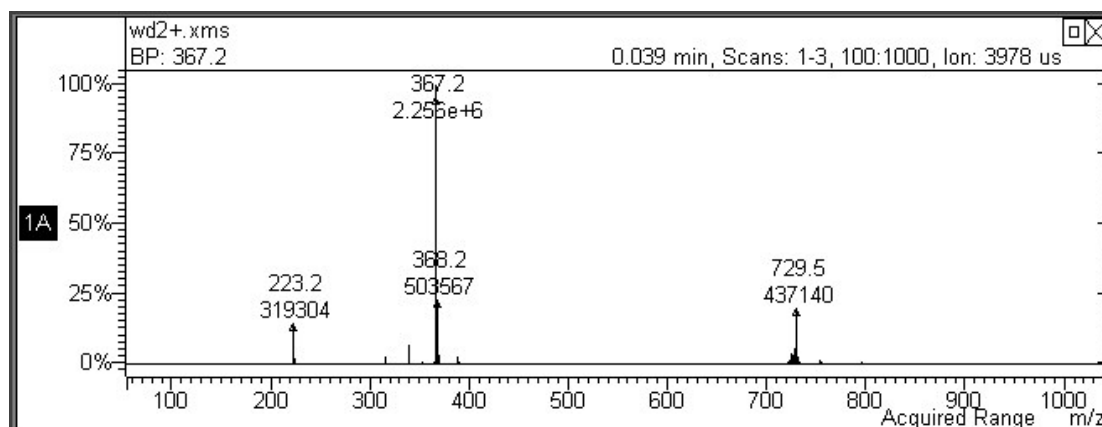
Pd(OAc)<sub>2</sub> (2.24 mg, 10.0 μmol), xantphos (5.79 mg, 10.0 μmol) and Cs<sub>2</sub>CO<sub>3</sub> (131 mg, 400 μmol) were dissolved in toluene (2 mL), then 2-Bromoquinoline (41.6 mg, 200 μmol) and 4-(dimethylamino)-*N*-phenylbenzamidinium (52.6 mg, 0.220 mmol) were added into toluene solution and the mixture was stirred at 140 °C for 24 h. The combined organic phase was concentrated and purified by flash column chromatography (petroleum ether/CH<sub>2</sub>Cl<sub>2</sub>/EtOAc, 2/1/0.5, v/v/v) to afford compound **2** as a white solid (67.4 mg, 92%). The <sup>1</sup>H NMR spectrum of **2** is shown in Figure S1. <sup>1</sup>H NMR (400 MHz, chloroform-*d*, room temperature): δ (ppm): 14.03 (s, 1H), 8.04 (d, *J* = 8 Hz, 1H), 7.87 (d, *J* = 8 Hz, 1H), 7.72 (d, *J* = 8 Hz, 1H), 7.58 (m, 3H), 7.38 (t, *J* = 8 Hz, 1H), 7.21 (t, *J* = 8 Hz, 2H), 7.01 (t, *J* = 8 Hz, 3H), 6.60 (d, *J* = 8 Hz, 2H), 2.97 (s, 6H). The <sup>13</sup>C NMR spectrum of **2** is shown in Figure S2. <sup>13</sup>C NMR (100 MHz, chloroform-*d*, room temperature) δ (ppm): 151.66, 137.05, 131.05, 129.24, 128.88, 127.38, 127.04, 125.30, 124.47, 122.92, 111.22, 40.16. LRESIMS is shown in Figure S3: *m/z* 367.2 [M + H]<sup>+</sup> (100%). HRESIMS: *m/z* calcd for [M + H]<sup>+</sup> C<sub>24</sub>H<sub>23</sub>N<sub>4</sub>, 367.1845; found 367.1853, error 2.2 ppm.



**Fig. S1**  $^1\text{H}$  NMR spectrum (400 MHz, chloroform-*d*, room temperature) of **2**.



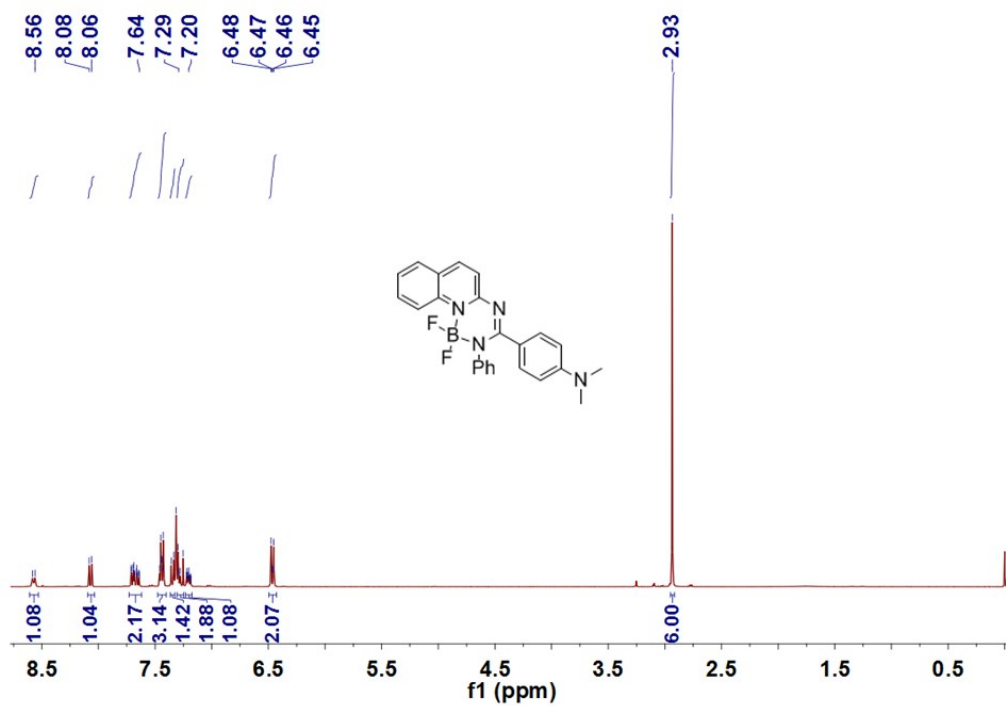
**Fig. S2**  $^{13}\text{C}$  NMR spectrum (100 MHz, chloroform-*d*, room temperature) of **2**.



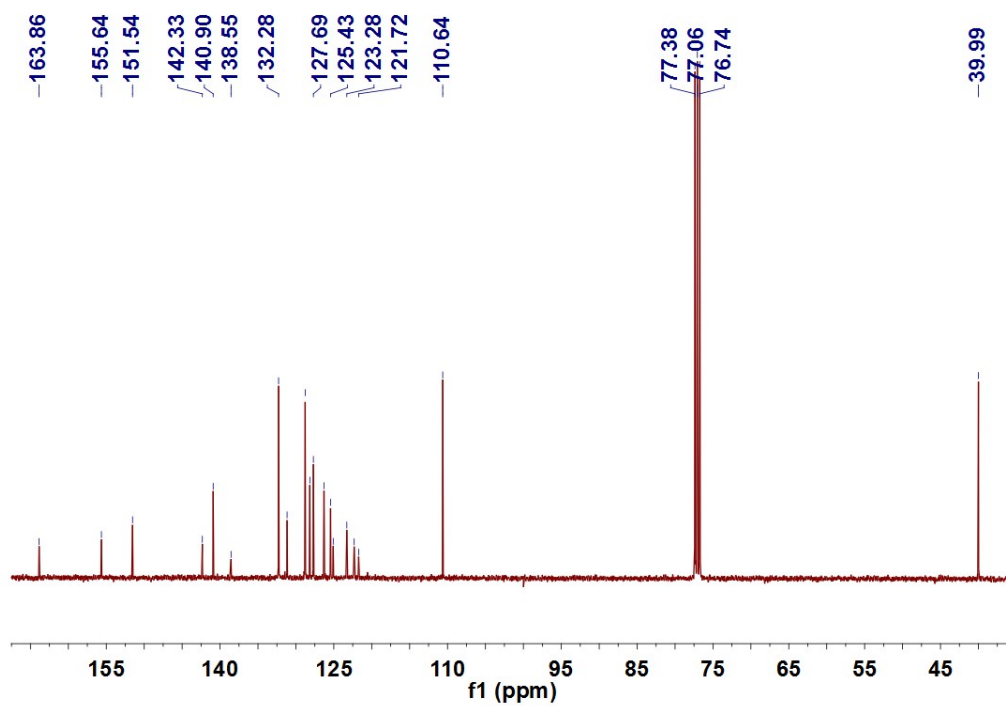
**Fig. S3** Electrospray ionization mass spectrum of **2**.

## 2.2 Synthesis of compound **P<sub>3</sub>T**

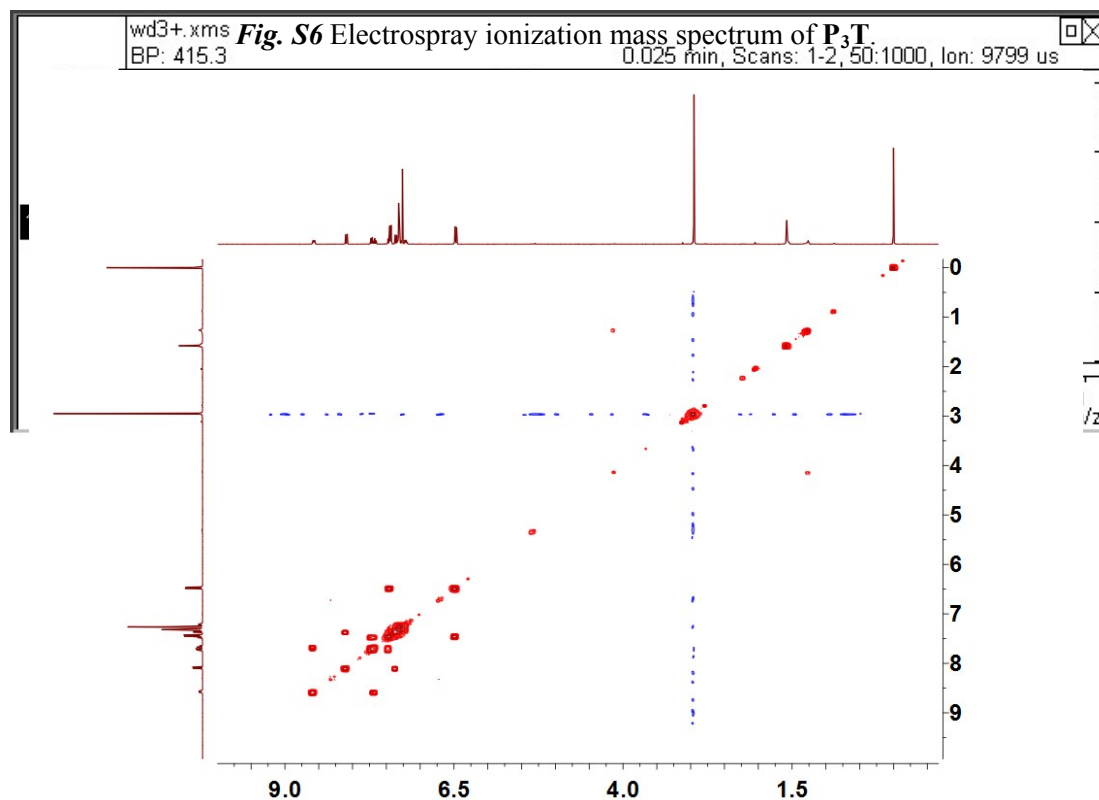
**2** (0.500 mmol) was dissolved in toluene (5 mL), then  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (2.00 mmol) and  $\text{Et}_3\text{N}$  (1.50 mmol) were added into toluene solution and the mixture was stirred at 120 °C for 6 h. The combined organic phase was concentrated and purified by flash column chromatography on neutral  $\text{Al}_2\text{O}_3$  to afford compound **P<sub>3</sub>T** as a green powder in the yield of 85%. The  $^1\text{H}$  NMR spectrum of **P<sub>3</sub>T** is shown in Figure S4.  $^1\text{H}$  NMR (400 MHz, chloroform-*d*, room temperature):  $\delta$  (ppm): 8.58 (d,  $J = 8$  Hz, 1H), 8.08 (d,  $J = 8$  Hz, 1H), 7.71–7.64 (m, 1H), 7.46–7.43 (m, 3H), 7.36–7.33 (m, 1H), 7.29–7.25 (m, 2H), 7.22–7.18 (m, 1H), 6.48–6.45 (m, 2H), 2.93 (s, 6H). The  $^{13}\text{C}$  NMR spectrum of **P<sub>3</sub>T** is shown in Figure S5.  $^{13}\text{C}$  NMR (100 MHz, chloroform-*d*, room temperature)  $\delta$  (ppm): 163.86, 155.64, 151.54, 142.33, 140.90, 138.55, 132.28, 131.16, 128.77, 128.16, 127.69, 126.69, 125.43, 125.08, 123.28, 122.31, 121.71, 110.64, 39.99. LRESIMS is shown in Figure S6:  $m/z$  415.3  $[\text{M} + \text{H}]^+$  (100%). HRESIMS:  $m/z$  calcd for  $[\text{M} + \text{H}]^+ \text{C}_{24}\text{H}_{22}\text{BF}_2\text{N}_4$ , 415.1828; found 415.1832, error  $-1.0$  ppm.



*Fig. S4*  $^1H$  NMR spectrum (400 MHz, chloroform-*d*, room temperature) of  $P_3T$ .

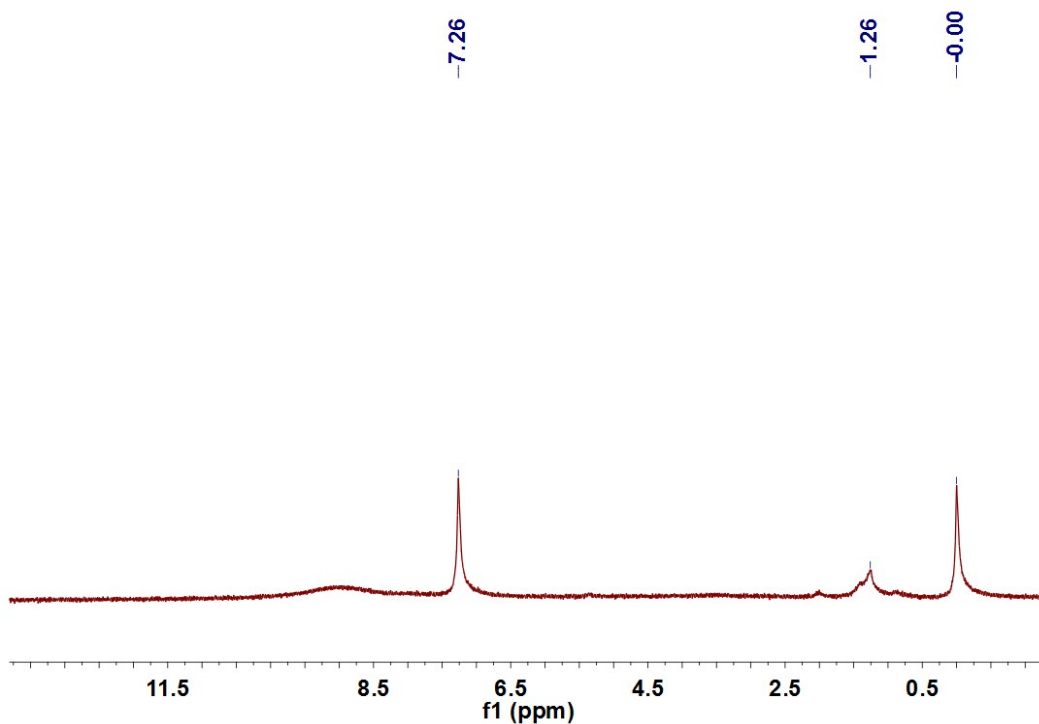


*Fig. S5*  $^{13}C$  NMR spectrum (100 MHz, chloroform-*d*, room temperature) of  $P_3T$ .

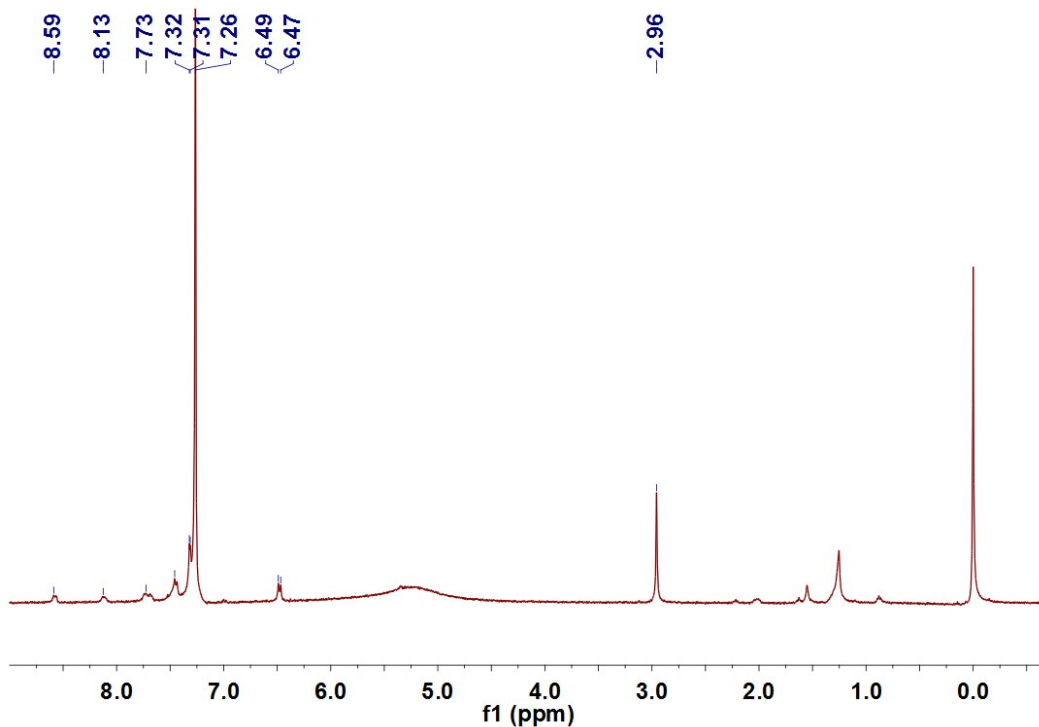


**Fig. S7** COSY (500 MHz, chloroform-*d*, room temperature) spectrum of **P<sub>3</sub>T**.

3. <sup>1</sup>HNMR spectra of **P<sub>3</sub>T** after acidification and alkalization



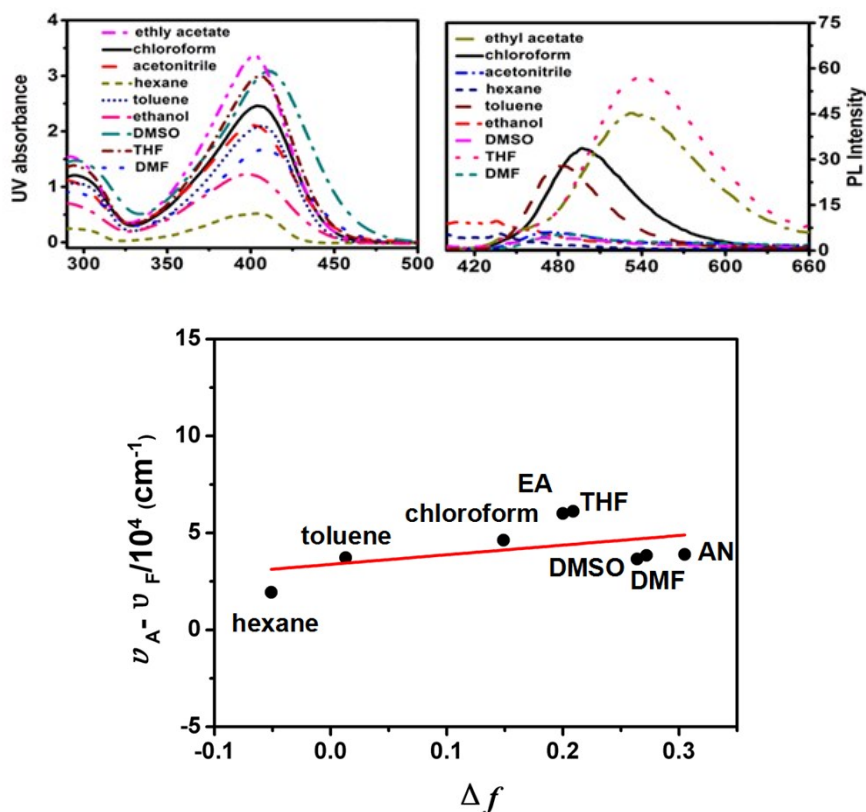
**Fig. S8**  $^1\text{H}$  NMR spectrum (400 MHz, chloroform-*d*, room temperature) of  $\text{P}_3\text{T}$  upon addition of TFA.



**Fig. S9**  $^1\text{H}$  NMR spectrum (400 MHz, chloroform-*d*, room temperature) of  $\text{P}_3\text{TH}^+$  upon addition of TEA.

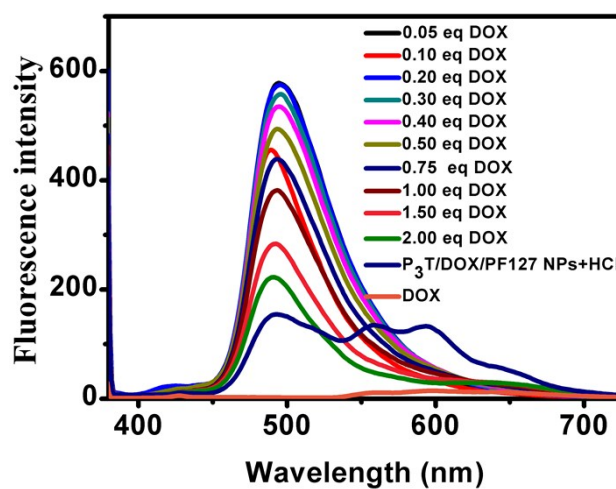
#### 4. Lippert–Mataga plot of $\text{P}_3\text{T}$





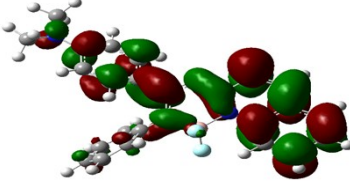
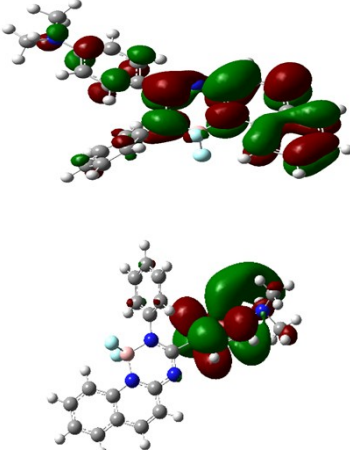
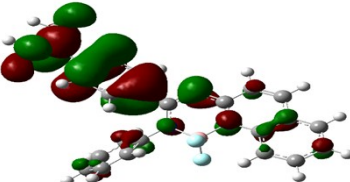
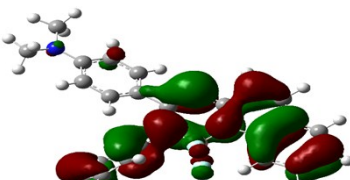
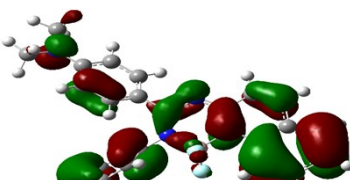
**Fig. S10** Lippert–Mataga plot  $P_3T$  in solvents with different polarities. Abbreviation: EA = ethyl acetate, THF = tetrahydrofuran, DMSO = dimethylsulfoxide, DMF = dimethylformamide, AN = acetonitrile.  $\nu_A$  = absorption wavelength,  $\nu_F$  = emission wavelength, and  $\Delta f$  = orientation polarizability =  $(\epsilon - 1)/(2\epsilon + 1) - (n^2 - 1)/(2n^2 + 1)$ , where  $\epsilon$  = dielectric constant and  $n$  = refractive index.

### 5. Fabrication of a ternary FRET system



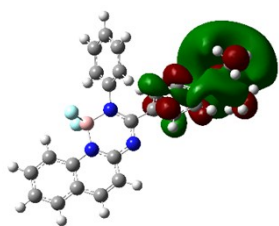

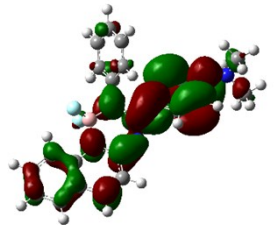
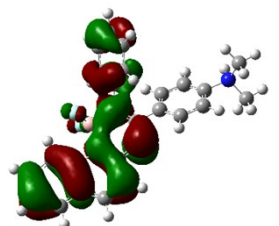
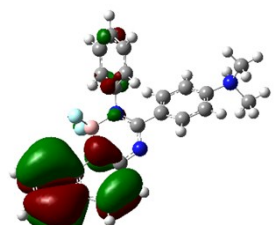
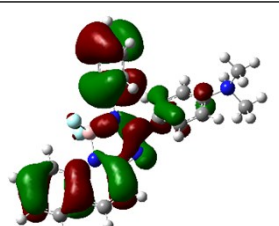
**Fig. S11** Fluorescence changes of  $P_3T$  in the presence of DOX.

6. Molecular orbital parameters of  $P_3T$

Species	Frontier Orbital	Energy Level/eV	Figure
$P_3T$	LUMO+1	-1.23	
	LUMO+1	-2.17-4.24	
	HOMO	-5.53	
	HOMO-1	-6.06	
	HOMO-2	-6.71	

*Table. S1* Molecular orbital parameters of  $P_3T$ .

7. Molecular orbital parameters of  $\text{P}_3\text{TH}^+$

Species	Frontier Orbital	Energy Level/eV	Figure
$\text{P}_3\text{TH}^+$	LUMO+2	-4.20	
	LUMO+1	-4.24	
	LUMO	-4.93	
	HOMO	-8.39	
	HOMO-1	-8.73	
	HOMO-2	-9.04	

**Table. S2** Molecular orbital parameters of  $\text{P}_3\text{TH}^+$ .

## *8. Preparation of nanoparticles*

### *8.1. Preparation of $P_3T$ /PF127 NPs*

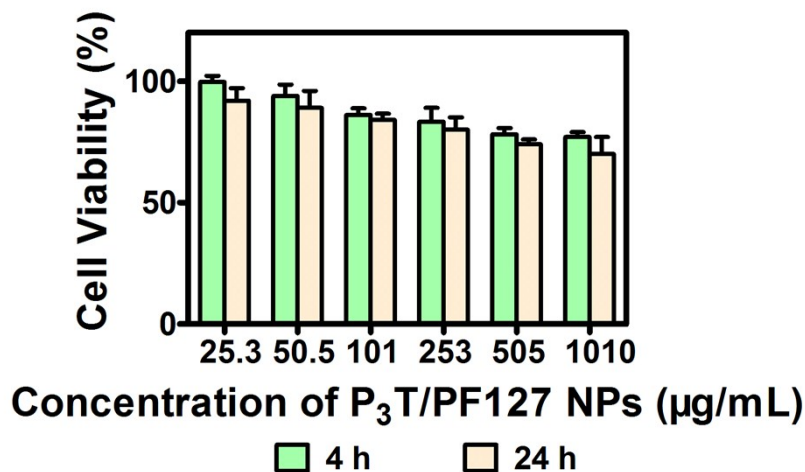
$P_3T$  (2.00 mg) was dissolved in THF (0.5 mL), PF127 (200 mg) was dissolved in water (5 mL). Then the THF solution containing  $P_3T$  was added into PF127 aqueous solution dropwisely. After stirring in the dark for 24 h, the resulting mixture was sealed in dialysis bags with a molecular weight cut-off of 8 kDa to 14 kDa and dialyzed against DI water for 24 h to remove free  $P_3T$ . The loading effect of  $P_3T$  was determined to be 0.86% by using UV spectroscopy. The morphology of the prepared  $P_3T$ /PF127 NPs was examined by TEM and DLS. TEM experiments were achieved on a HT-7700 instrument. Afterwards, the solution of  $P_3T$ /PF127 NPs rested overnight before being used for DLS tests. The diameters of the  $P_3T$ /PF127 NPs were measured on a Nano-ZS ZEN3600 instrument.

### *8.2. Preparation of $P_3T$ /DOX/PF127 NPs*

$P_3T$  (2.00 mg) was dissolved in THF (0.5 mL), DOX·HCl (3.00 mg) was dissolved in water (0.5 mL) in the presence of triethylamine (TEA). Then the THF solution containing  $P_3T$  and DOX aqueous solution was added into PF127 (200 mg, 5 mL) aqueous solution dropwisely. After stirring in the dark for 24 h, the resulting mixture was sealed in dialysis bags with a molecular weight cut-off of 8 kDa to 14 kDa and dialyzed against DI water for 24 h to remove free  $P_3T$  and DOX in the solution.

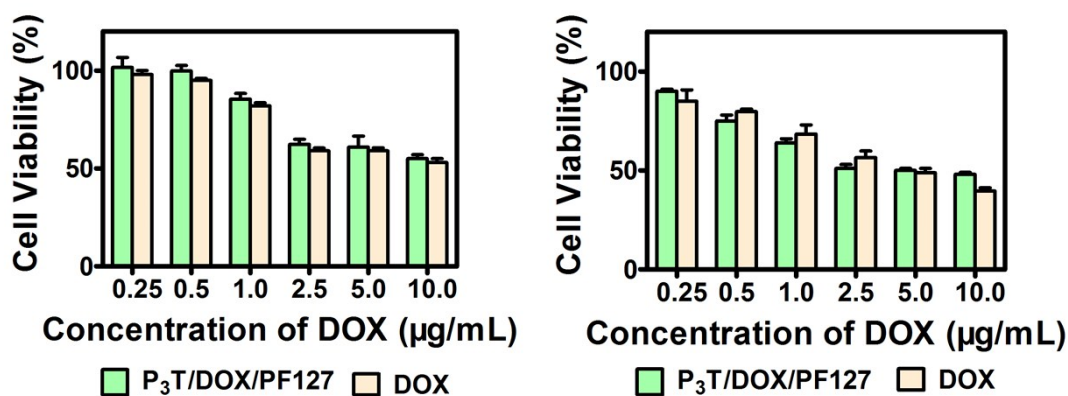
## *9. Cytotoxicity Evaluations*

The cytotoxicity of free DOX,  $P_3T$ /PF127 NPs, and  $P_3T$ /DOX/PF127 NPs was evaluated by using MTT assay. HeLa cells (human cervical carcinoma, ATCC) were seeded in 96-well plates at  $1 \times 10^4$  cells/well, allowed to adhere overnight and incubated with serum-free culture media containing free DOX,  $P_3T$ /PF127 NPs, or  $P_3T$ /DOX/PF127 NPs at a series of concentration. After 4 h or 24 h incubation, the media was removed and washed with PBS for three times. The cells were incubated in 100  $\mu$ L DMEM medium containing 0.5 mg/mL MTT reagent for an additional 4 h, 100  $\mu$ L DMSO was added to each well to dissolve formazan crystal formed. Eventually, each well was measured using a scanning spectrophotometer (Model 550, Bio-Rad) at a wavelength 570 nm.



**Fig. S12** Cytotoxicity experiment of P<sub>3</sub>T/PF127 NPs at various concentrations on HeLa cells for 4 h and 24 h.

As shown in Figure S12, we found the relative cell viability was higher than 75% by culturing HeLa cells with of P<sub>3</sub>T/PF127 NPs for 4 h and 24 h. The concentration in the MTT assay was extremely high (1010 µg/mL), and the viability reached higher than 75%, confirming low cytotoxicity of P<sub>3</sub>T/PF127 NPs.



**Fig. S13** Cytotoxicity experiment of P<sub>3</sub>T/DOX/PF127 NPs and free DOX at various concentrations on HeLa cells for 4 h (left) and 24 h (right).

### 10. Controlled Release Studies

*In vitro* released profile of the DOX from P<sub>3</sub>T/DOX/PF127 NPs was monitored using the dialysis method. The P<sub>3</sub>T/DOX/PF127 ternary complex (3 mg) was dissolved in 2 mL phosphate buffer solutions at pH values of 5.0, 6.0 or 7.4, sealed in dialysis bags with a molecular weight cut-off of 8 kDa to 14 kDa. The dialysis apparatus was agitated on an orbital shaker at 100 rpm at 37 °C. At designated time intervals, 1 mL medium was taken out

from the 25 mL solution out of dialysis bag for UV detection and was then put back to original system. The UV absorption was measured on a UV spectrophotometer (UV-2550, Shimadzu, Japan) at the wavelength of 480 nm. The DOX concentration was calculated with a standard curve calibrated with DOX samples of known concentration. The DOX concentration in the solution was calculated based on the standard curve calibrated with DOX solution of known concentration.

### 11. X-ray crystal data of $P_3T$

Crystallographic data: yellow,  $C_{24}H_{21}BF_2N_4$ ,  $FW$  414.26, triclinic, space group  $P\bar{1}$ ,  $a = 8.4892(7)$ ,  $b = 8.7454(7)$ ,  $c = 14.2585(11)$  Å,  $\alpha = 83.179(7)^\circ$ ,  $\beta = 79.851(7)^\circ$ ,  $\gamma = 84.564(7)^\circ$ ,  $V = 1031.68(14)$  Å<sup>3</sup>,  $Z = 2$ ,  $D_c = 1.334$  g cm<sup>-3</sup>,  $T = 293.15$  K,  $\mu = 0.092$  mm<sup>-1</sup>, 6450 measured reflections, 3780 independent reflections, 282 parameters, 0 restraints,  $F(000) = 432$ ,  $R_1 = 0.0773$ ,  $wR_2 = 0.1357$  (all data),  $R_1 = 0.0491$ ,  $wR_2 = 0.1119$  [ $I > 2\sigma(I)$ ], max. residual density 0.153 e<sup>-</sup>Å<sup>-3</sup>, and goodness-of-fit ( $F^2$ ) = 1.041. CCDC: 1428967.

### References:

- S1. D. B. Zhao, G. C. Li, D. Wu, X. R. Qin, P. Neuhaus, Y. Y. Cheng, S. J. Yang, Z. Y. Lu, X. M. Pu, C. Long and J. S. You, *Angew. Chem. Int. Ed.*, 2013, **52**, 13676.