Electronic Supplementary Material (ESI) for Materials Chemistry Frontiers.

Electronic Supporting Information

Efficient red luminogen with aggregation-induced emission for in vivo three-photon brain vascular imaging

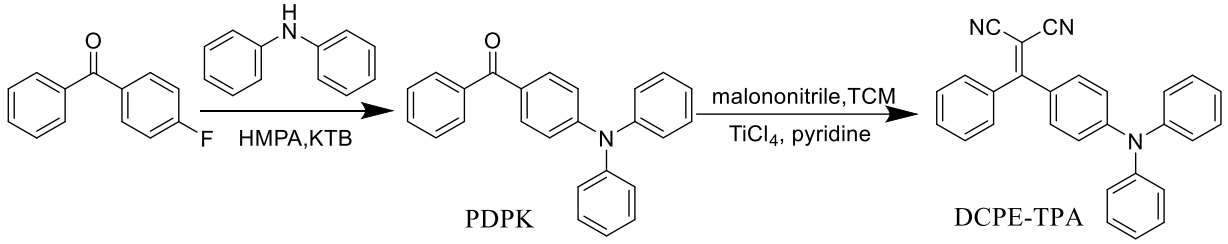
Haiyan Tian,‡[a] Dongyu Li,‡[b] Xi Tang,[a] Yubo Zhang,[a] Zhiyong Yang,[c] Jun Qian, *[b] Yong Qiang Dong*[a] and Mei Han*[a]

[a]Beijing Key Laboratory of Energy Conversion and Storage Materials, Key Laboratory of Radiopharmaceuticals, Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, China, E-mail: dongyq@bnu.edu.cn; hanmei@bnu.edu.cn.

[b]State Key Laboratory of Modern Optical Instrumentations, Centre for Optical and Electromagnetic Research, College of Optical Science and Engineering, Zhejiang University, Hangzhou 310058, China, E-mail: qianjun@zju.edu.cn.

[c]PCFM Lab, GDHPPC Lab, Guangdong Engineering Technology, Research Center for High-performance Organic and Polymer Photo-electric, Functional Films, State Key Laboratory of School of Chemistry, Sun Yat-Sen University, Guangzhou 510275, China

Synthetic routes



Scheme S1. Synthetic routes to compound DCPE-TPA.

***Synthesis of phenyl-4-diphenylaminophenylketone (PDPK)*:** In a two-neck flask, diphenylamine (8.5 g, 50 mmol) and potassium tert-butoxide (5.85 g, 52 mmol) were dissolved in 25 mL of HMPA under an argon atmosphere. The mixture was heated to 60 ℃ for 2 h. Then, 4-fluorophenylmethanone (4.00 g, 20 mmol) dissolved in 10 mL of HMPA was slowly added into the flask. The reaction mixture was heated to 120 ℃ for 6 h. After cooling to room temperature overnight, pale yellow solid precipitates were filtered off and washed with hexane. The crude product was separated by silica gel column chromatography by means of ethyl acetate/petroleum ether. Finally, a yellow solid was obtained with a yield of 70.0%. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.80-7.78 (d, 2H), 7.74-7.72 (d, 2H), 7.56-7.52 (t, 1H), 7.48-7.44 (t, 2H), 7.35-7.31 (t, 4H), 7.20-7.12 (m, 6H), 7.05-7.02 (d, 2H).

Synthesis of 1,1-dicyano-2-phenyl-2-(4-diphenylamino) phenylethylene (DCPE-TPA): PDPK (3.37 g, 10 mmol) and malononitrile (2.11 g, 32 mmol) were added to a 250 mL two-neck flask. The mixture was dissolved in 60 mL of anhydrous chloroform under an argon atmosphere. Then, 4.4 mL (40 mmol) of Titanium tetrachloride ($TiCl_4$) was slowly added into the flask at 0 ℃. Pyridine (5 mL) was then added to the reaction mixtures and stirring continued for 3.5 h at room temperature. The reaction was then quenched with an excess of distilled water and then extracted with a large volume of chloroform. The organic layer was washed twice with distilled water, dried over $MgSO_4$ and concentrated by a rotary evaporator. The crude product was purified by a silica gel column using a mixture of ethyl acetate and petroleum ether as the eluent. A red powder was obtained with a yield of 80.0%. The purified product was characterized using spectroscopic methods. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.57-7.53 (t, 1H), 7.49-7.41 (m, 4H), 7.37-7.31 (q, 6H), 7.20-7.12 (t, 6H), 6.93-6.91 (d, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, δ): 173.56, 152.39, 145.65, 136.70, 132.71, 132.02, 130.51, 129.85, 128.71, 126.63, 126.56, 125.58, 118.32, 115.26, 114.94. HRMS (ESI) *m*/*z*: $[M+H]^+$ calcd for $C_{28}H_{20}N_3$, 398.1613; found, 398.1652. Anal. calcd for $C_{28}H_{19}N_3$: C 84.61, H 4.82, N 10.57; found: C 84.50, H 4.89, N 10.11.

Cell culture

HeLa cells were cultured in DMEM containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in a humidified atmosphere of 95% air and 5% CO_2 at 37 ℃. The medium was changed three times per week. DCPE-TPA NPs at a concentration of 0.1 mg mL^{-1} (250 μM) were prepared first and then diluted by adding 40 μL of the stock solution into 2 mL of culture medium. The live cells were stained with such medium for 3 h, and the excess dye was washed away with phosphate-buffered saline (PBS). The cells were subsequently imaged using a Nikon $A1^+$ confocal laser scanning

microscope.

Cytotoxicity study

The cytotoxicity of the DCPE-TPA NPs on HeLa cells was evaluated by a 3-(4,5-dimethyl-2-thiazolyl)-2,5diphenyltetrazolium bromide (MTT) assay. The viability of the cells was assayed using an MTT kit and the absorbance at 570 nm was detected by a Victor^3 V Multilabel reader (PerkinElmer, Waltham, MA, USA). Cells were seeded at 5000 cells per well in a 96-well plate and cultured overnight. DCPE-TPA NP solutions with different concentrations were then added to the 96-well plate. After treatment for 24 h, the cells were photographed under an inverted microscope with a 40x objective and then the medium was removed followed by washing the cells with PBS. The medium was changed and incubated with an MTT solution at 5 (mg mL^{-1})/well for 24 h at 37 ℃. Then, 150 μL of DMSO was added to dissolve the purple crystals. Then, the absorbance of MTT at 570 nm was detected by a plate reader. The cell viability was expressed as the ratio of the absorbance of the cells incubated with various concentrations of DCPE-TPA NPs to that of the cells incubated with culture medium. Each of the experiments was performed at least 3 times.

Three-photon fluorescence microscopic imaging system

An upright scanning microscope (Olympus, BX61W1-FV1200) equipped with a 1560 nm fs laser (FLCPA-01C, Calmar Laser, 1 MHz, 400 fs) was adopted for three-photon microscopic imaging. The 1560 nm fs laser beam was focused onto the mouse brain with a water-immersed objective lens (XLPlan N, 25 ×, NA = 1.05, working distance = 2.0 mm), which had a large NA and good NIR transmission. The excited fluorescence signal was then collected by the same objective lens. After passing through a dichroic mirror (980 SP) and a 590 nm longpass filter, the fluorescence signal was collected by a photomultiplier tube (HPM-100-50, Becker & Hickl GmbH). It should be noticed that, the objective was immersed in D_2O instead of H_2O during the imaging process, in order to avoid the absorption to the 1560-nm excitation source.

Animal treatments

Female ICR mice (8 weeks old) were used as a biological model. The mice were anesthetized and their scalps and skulls were removed through microsurgery. A small smooth metal ring with a handle was then mounted onto the exposed brain of each mouse. Afterwards, a round thin glass coverslip was embedded in the ring and adhered to each mouse with dental cement to protect the brain, as well as provide a cranial window. The metal ring was then connected to a heavy metal plate to immobilize each mouse during imaging. Finally, a PBS (1×) dispersion of DCPE-TPA NPs (200 μL, 0.2 mg mL^{-1}) was intravenously injected into the blood system of each mouse through the tail vein for three-photon fluorescence microscopic imaging of the vasculature in the brain. All the animal experiments were carried out in compliance with the Zhejiang University Animal Study Committee's requirements for the care and use of laboratory animals in research.

Table S1 Crystal data and structure refinement parameters for **1**OC and **1**RC**.**

Crystals names	**1**OC	**1**RC
Empirical formula	$C_{28}H_{19}N_3$	$C_{56}H_{38}N_6$
Formula weight	397.46	794.92
Temperature/K	100.01(10)	100.01(10)
Crystal system	orthorhombic	orthorhombic
Space group	$P2_12_12_1$	Pca21
a/Å	8.12125(15)	28.5615(4)
b/Å	8.87819(17)	8.12800(10)
c/Å	29.0812(5)	17.5584(2)
α/°	90	90
β/°	90	90
γ/°	90	90
Volume/Å^3	2096.81(7)	4076.15(9)
Z	4	4
ρ_{calc} g/cm^3	1.259	1.295
μ/mm^{-1}	0.582	0.598
F(000)	832.0	1664.0
Crystal size/mm^3	0.3 × 0.1 × 0.1	0.3 × 0.12 × 0.1
Radiation	CuKα (λ = 1.54184)	CuKα (λ = 1.54184)
2Θ range for data collection/ °	10.418 to 146.806	7.98 to 147.468
Index ranges	-6 ≤ h ≤ 9, -10 ≤ k ≤ 10, -29 ≤ l ≤ 35	-35 ≤ h ≤ 31, -10 ≤ k ≤ 9, -21 ≤ l ≤ 21
Reflections collected	7175	31408
Independent reflections	4071 [R_{int} = 0.0424, R_{sigma} = 0.0541]	8064 [Rint = 0.0577, Rsigma 0.0428]
Data/restraints/parameters	4071/0/280	8064/1/559
Goodness-of-fit on F^2	1.033	1.040
Final R indexes [I>=2σ (I)]	R_1 = 0.0380, wR_2 = 0.0976	R1 = 0.0371, wR2 = 0.0920
Final R indexes [all data]	R_1 = 0.0392, wR_2 = 0.0987	R1 = 0.0396, wR2 = 0.0949
Largest diff. peak/hole / e Å^{-3}	0.14/-0.19	0.18/-0.27
Flack parameter	0.0(4)	0.5(3)

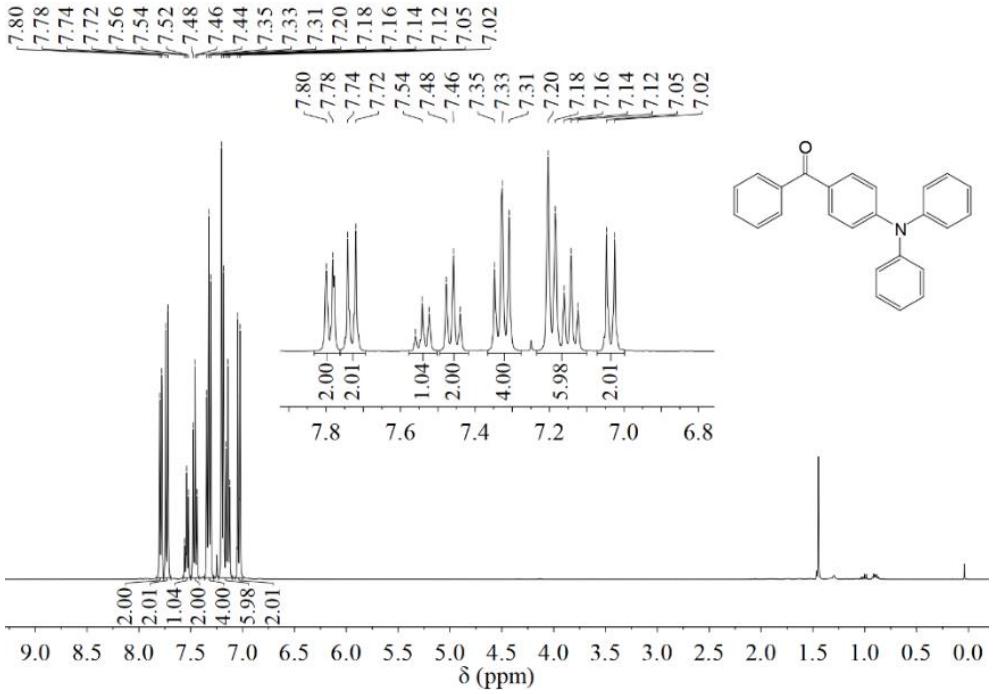


Fig. S1 The ^{1}H NMR spectrum of compound PDPK in $CDCl_3$ solvent.

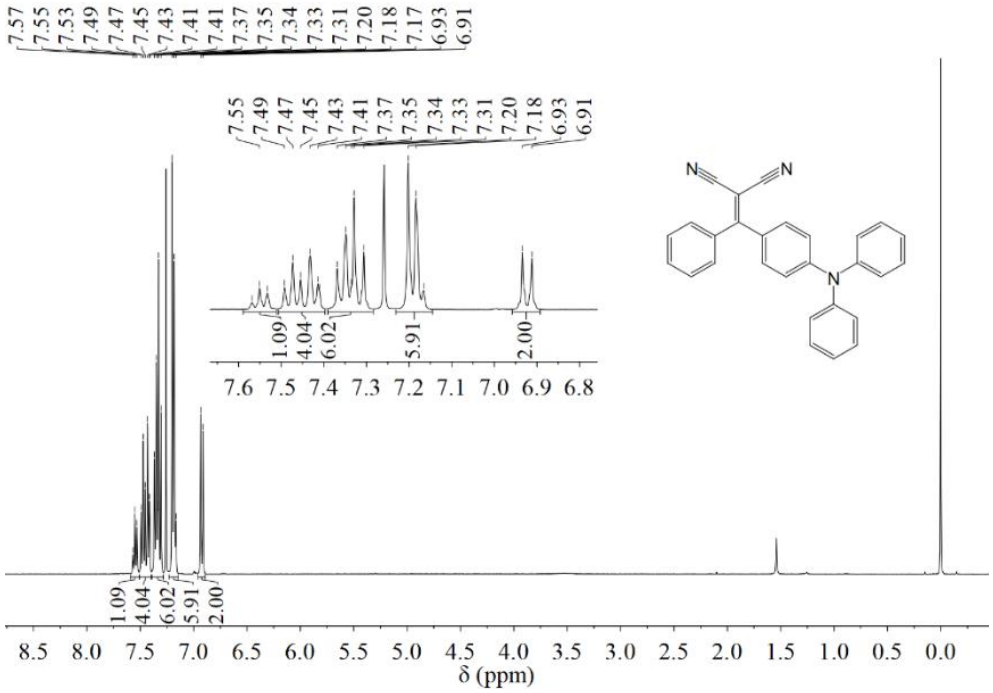


Fig. S2 The ^{1}H NMR spectrum of compound DCPE-TPA in $CDCl_3$ solvent.

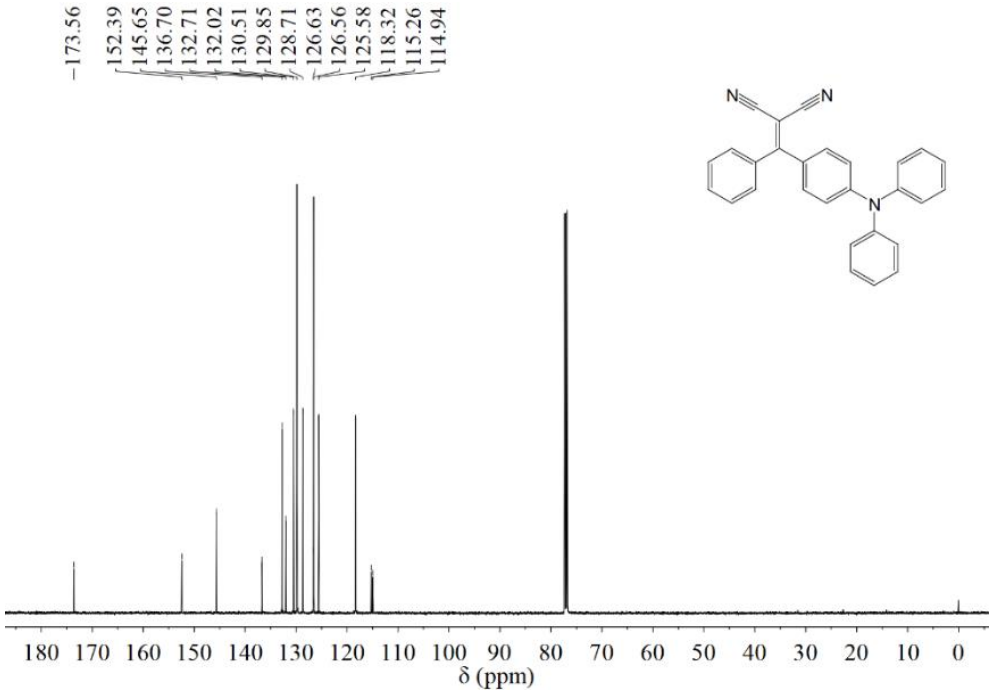


Fig. S3 The ^{13}C NMR spectrum of compound DCPE-TPA in $CDCl_3$ solvent.

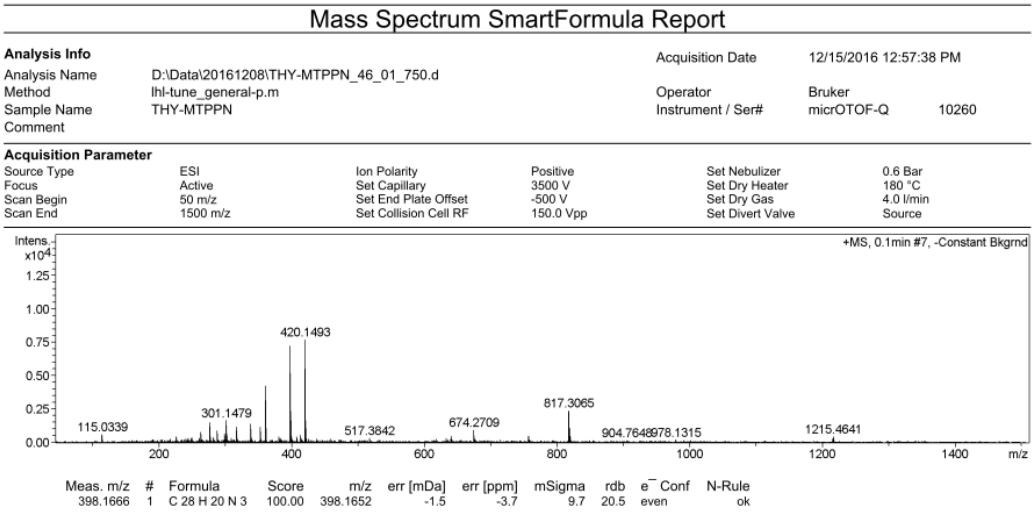


Fig. S4 The HRMS spectrum of compound DCPE-TPA.

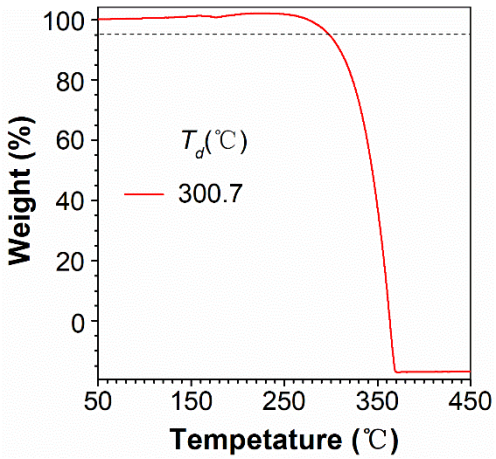


Fig. S5 TGA thermogram of compound DCPE-TPA recorded at a heating rate of 10 K/min.

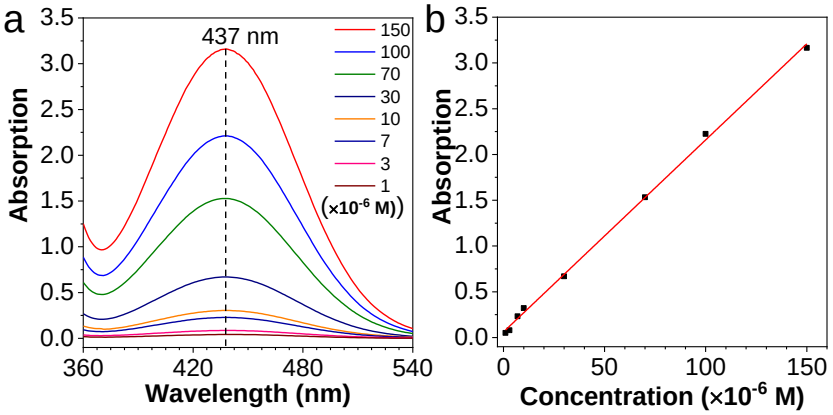


Fig. S6 (a) The UV-Vis absorption spectra of DCPE-TPA in dichloromethane with different concentration; (b) linear fitting about absorption intensity versus concentration (10^{-6} M), $R^2 = 0.998$.

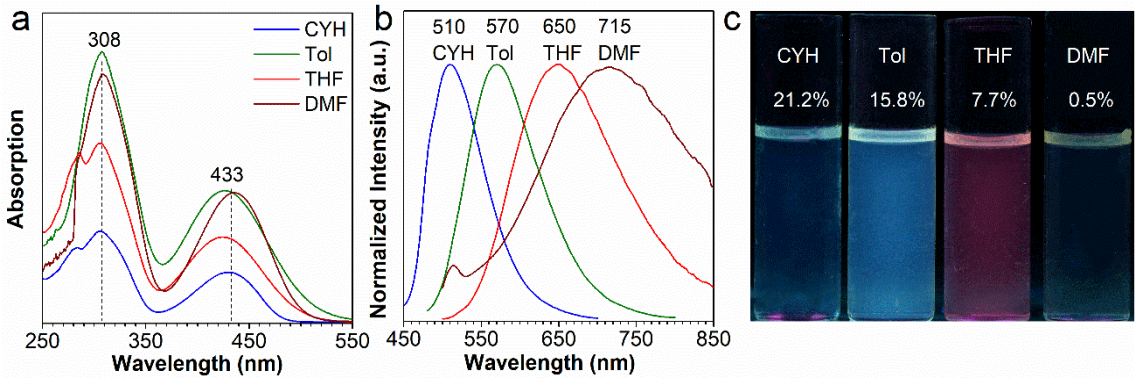


Fig. S7 (a) UV-Vis absorption and (b) normalized photoluminescence emission spectra in cyclohexane (CYH), toluene (Tol), tetrahydrofuran (THF) and dimethyformamide (DMF); (c) photographs of DCPE-TPA in solvents taken under 365 nm UV illumination, exposure time: 1 s.

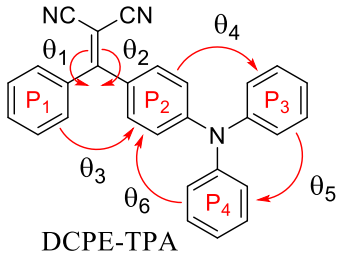


Table S2 Dihedral angle of **1**OC and **1**RC. θ_1, dihedral angle between benzene ring P_1 and double bond plane; θ_2, dihedral angle between benzene ring P_2 and double bond plane; θ_3, dihedral angle between benzene ring P_1 and benzene ring P_2; θ_4, dihedral angle between benzene ring P_2 and benzene ring P_3. θ_5, dihedral angle between benzene ring P_3 and benzene ring P_4; θ_6, dihedral angle between benzene ring P_2 and benzene ring P_4.

Crystal	λ_{em} (nm)	θ_1	θ_2	θ_3	θ_4	θ_5	θ_6
1OC	578	43.74	30.45	55.92	77.40	74.91	55.68
1RC-1	610	41.85	35.11	62.27	65.90	75.21	53.78
1RC-2		36.57	34.79	55.64	58.48	68.91	58.72

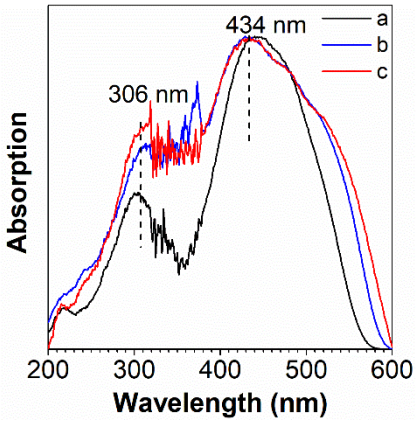


Fig. S8 UV-Vis reflectance spectra of (a) **1**OC, (b) **1**RC and (c) **1**Am.

Table S3 Summarization of the Intermolecular Interactions in the Crystals of **1**OC

Interactions	d /Å [a)] (N) [b)]	A/ °[c)]
1C-H ··N	2.589(4)	163.350(128)
2C-H ··N	2.822(3)	141.729(124)
3C-H ··N	2.990(3)	157.245(115)
4C-H ··π	2.780(2)	147.151(111)
5C-H ··π	2.803(2)	139.911(125)
6C-H ··π	2.973(2)	136.895(117)
7C-H ··π	3.104(2)	134.323(133)
8C-H ··π	3.146(2)	167.588(136)
9C-H ··π	3.478(2)	145.802(132)
10C-H ··π	3.492(2)	132.063(109)

a) Distance of C-H···π or C-H ··N interaction. b) Number of the intermolecular interactions. c) Angel of C-H···π or C-H ··N interaction.

Table S4 Summarization of the Intermolecular Interactions in the Crystals **1**RC.

Interactions	d /Å [a)] (N) [b)]	A/ °[c)]
1C-H ··N	2.527(2)	172.115(150)
2C-H ··N	2.646(2)	130.031(161)
3C-H ··N	2.676(1)	167.713(151)
4C-H ··N	2.857(2)	137.510(165)
5C-H ··N	2.874(2)	140.678(182)
6C-H ··N	2.882(1)	128.813(150)
7C-H ··π	2.725(2)	152.897(154)
8C-H ··π	2.860(1)	139.585(148)
9C-H ··π	2.885(1)	167.267(146)
10C-H ··π	2.966(2)	158.557(189)
11C-H ··π	3.123(1)	125.427(141)
12C-H ··π	3.163(2)	139.131(153)
13C-H ··π	3.212(2)	134.047(154)

14C-H ··π	3.215(1)	122.774(141)
15C-H ··π	3.257(1)	131.722(156)
16C-H ··π	3.285(1)	121.270(147)
17C-H ··π	3.304(2)	133.873(148)
18C-H ··π	3.384(1)	125.903(152)
19C-H ··π	3.391(2)	147.156(154)
20C-H ··π	3.505(2)	129.187(154)
21C-H ··π	3.571(2)	122.080(148)

a) Distance of C-H···π or C-H ··N interaction. b) Number of the intermolecular interactions. c) Angel of C-H···π or C-H ··N interaction.

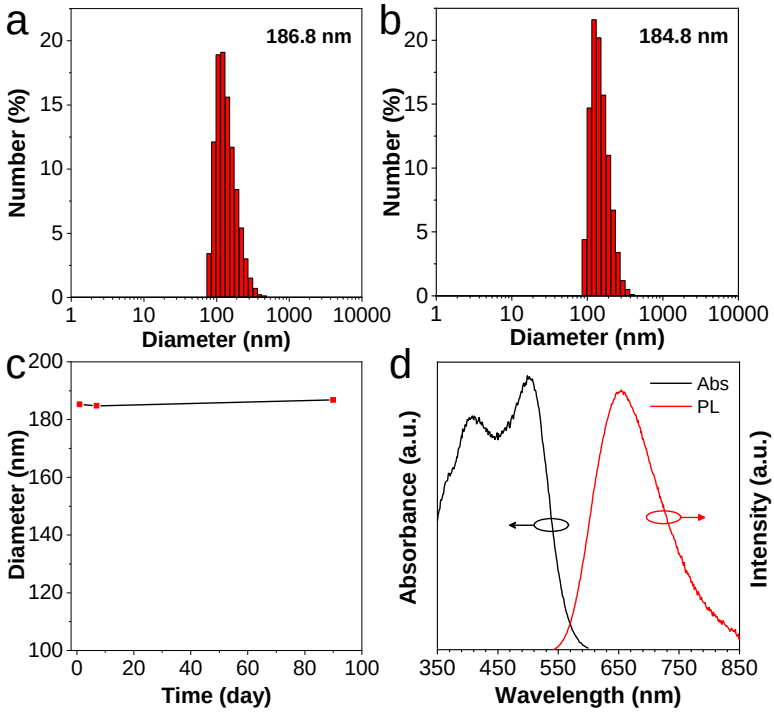


Fig. S9 DLS of DCPE-TPA NPs after stored at 4 ℃ for (a) 7 days and (b) 90 days; (c) size measurement of DCPE-TPA NPs at different time interval; (d) UV absorption and PL spectra of the DCPE-TPA NPs after stored at 4 ℃ for 90 days.

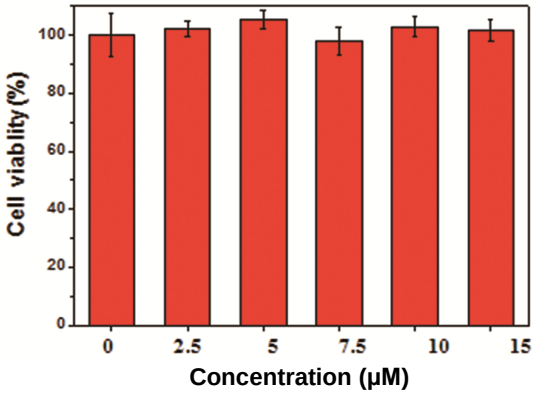


Fig. S10 Concentration-dependent cytotoxicity to HeLa cells incubated with different concentrations of DCPE-TPA NPs for 24 h.

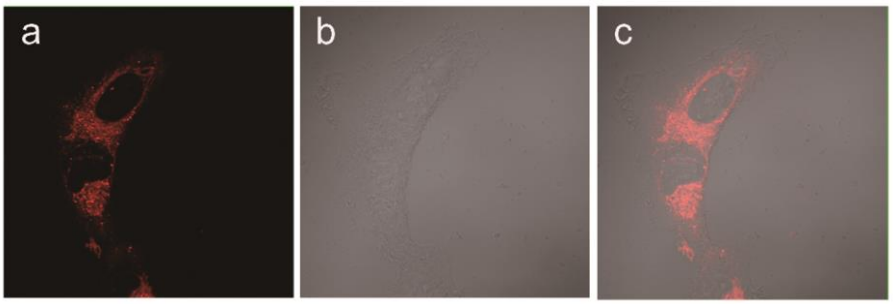


Fig. S11 CLSM images of HeLa cells incubated with 5 μM of DCPE-TPA NPs for 3 h. (a) Excited with a 488 nm laser, (b) bright field image and (c) merged image of (a) and (b). Scale bar = 20 μm.