

Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B

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Electronic Supplementary Information (ESI) for

Modulating Aggregation-Induced-Emission via

Non-Conjugated Linkage of Fluorophores to

Tetraphenylethenes

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Experimental Details

Materials. All manipulations were carried out under a nitrogen atmosphere using standard Schlenk techniques, unless otherwise stated. All starting materials were obtained commercially as analytical-grade and used without further purification. **Bu-DNS** and **Bu-NBD** was synthesized according to the previous literature.^{S1}

Characterizations. ¹H and ¹³C NMR spectra were collected on an American Varian Mercury Plus 400 spectrometer (400 MHz). Mass spectra were recorded with the EI-MS spectrometer. UV-Vis spectra were recorded using a Hitachi U-3310 visible recording spectrophotometer. Fluorescence spectra were recorded using a Perkin Elmer LS-55. Dynamic light scattering (DLS) measurements were performed on the Zetasizer instrument ZEN3600 (Malvern, UK) with a 173 back scattering angle and He-Ne laser (633 nm). Transmission electron microscopy (TEM) studies were performed on a LIBRA 200 FE electron microscope with an accelerating voltage of 200 KV. Crystal-structures of **TPE-NBD** and **TPE-DNS** were obtained on a Bruker APEX DUO CCD system via single crystal X-ray diffraction experiments. Absolute fluorescence quantum yields were measured on a Hamamatsu C11347 Absolute PL quantum yield spectrometer.

Synthesis of TPE-DNS. Compounds **DNS-Cl** (67.4 mg, 0.25 mmol) and **TPE-MA** (108.5 mg, 0.3 mmol) were added into a 50 mL round-bottomed flask, followed by the addition of DCM (20 mL) and Et₃N (0.5 mL) under nitrogen atmosphere. The reactants were stirred at room temperature for 12 h. The mixture was then evaporated and the residue was purified with column chromatography. A green solid (79 mg) was obtained, yield: 53%. ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 2.87 (s, 6H), 3.94 (d, J=4 Hz, 2H), 4.71 (t, J = 8Hz, 1H), 6.77-6.84 (m, 5H), 6.90-6.96 (m, 5H), 7.05 (br, 8H), 7.14-7.16 (d, J=8 Hz, 1H), 7.46-7.54 (m, 2H), 8.22 (t, J=6 Hz, 2H), 8.5 (d, J=8 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃): 45.56, 47.21, 115.16, 118.55, 123.07, 126.37, 127.01, 127.54, 128.34, 129.75, 130.46, 131.09, 131.37, 134.03, 140.03, 141.09, 143.31, 151.86. EI-MS: m/z = 594.13. Calculated exact mass: 594.23.

Synthesis of TPE-NBD. Compounds **NBD-Cl** (80.0 mg, 0.4 mmol) and compound **TPE-MA** (159.1 mg, 0.44 mmol) were added into a 50 mL round-bottomed flask, followed by the addition of MeCN (20 mL) and Et₃N (0.2 mL) under nitrogen atmosphere. The reactants were stirred at room temperature for 5 h. The mixture was then evaporated and the residue was purified with column chromatography. A red solid (62 mg) was obtained, yield: 30%. ¹H NMR (400 MHz, CD₃CN): δ (ppm) = 4.64 (s, 2H), 6.15 (d, J=4 Hz, 2H), 7.01-7.03 (m, 8H), 7.10-7.17 (m, 11H), 7.86 (br, 1H),

8.40-8.42 (d, J=8 Hz, 1H). ¹³C NMR (100 MHz, CDCl₃): 144.23, 143.27, 143.11, 141.75, 141.65, 139.77, 136.03, 132.60, 132.05, 131.09, 127.66, 126.92, 126.52, 99.23, 77.36, 77.04, 76.73, 47.99. EI-MS: m/z = 524.04. Calculated exact mass: 524.18.

Crystallographic Details

Single crystals of **TPE-NBD** and **TPE-DNS** suitable for crystallographic analysis were obtained by diffusing hexane into dichloromethane solution of **TPE-NBD** and **TPE-DNS** at room temperature, respectively. These crystals were mounted on a glass fiber for diffraction experiments. Intensity data were collected on a Nonius Kappa CCD diffractometer with Mo K α radiation (0.71073 Å) at room temperature. The structures were solved by a combination of direct methods (SHELXS-97)^{S2} and Fourier difference techniques and refined by full-matrix least-squares (SHELXL-97).^{S3} All non-H atoms were refined anisotropically. The hydrogen atoms were placed in the ideal positions and refined as riding atoms. Further crystal data are summarized in Tables S2 and S3. Crystallographic data for **TPE-DNS** and **TPE-NBD** in this paper have been deposited in the Cambridge Crystallographic Data Centre as supplemental publication CCDC 1548388 and 1548389.

Measurement of Absolute fluorescence quantum yields

Stock acetonitrile solutions of **TPE-DNS** and **TPE-NBD** (0.1 mM) were firstly prepared. After diluting 1 mL of the stock solution with a calculated amount of acetonitrile in a 10 mL volumetric flask, water was added dropwise under vigorous stirring to furnish 10 μ M of **TPE-DNS** and **TPE-NBD** solution, respectively. Water fractions (f_w) in the final acetonitrile/water mixtures amounted to 0, 60 and 90 vol %, respectively. Absolute fluorescence quantum yields of these samples were determined using a Hamamatsu C11347 Absolute PL quantum yield spectrometer.

Cell Growth

HeLa cells were grown in dulbecco's minimum essential medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum and maintained in an incubator at 37 °C in a 5% CO₂ environment.

Cytotoxicity Assay

HeLa cells were seeded into a 96-well plate, maintained overnight in DMEM containing 10% FBS, and then treated with **TPE-DNS** and **TPE-NBD** at 37 °C for 24 h. After 20 μ L of freshly prepared

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL in 1× phosphate-buffered saline (PBS) was added to each well, the wells were incubated for 4 h. The supernatant was removed, and the cells were lysed by addition of 100 µL of DMSO per well and then photographed under a microscope.

Cell Imaging

HeLa cells were grown in DMEM containing 10% FBS, and 100 000 cells were then seeded on 35 mm culture plates, which were incubated at 37 °C for 24 h. 1 µL of TPE-DNS and TPE-NBD stock solution (5 mM) were added to 1 mL of medium containing HeLa cells in a 20-mm plate for staining. The stock solution was prepared by in DMSO. After incubation at 37 °C for different time, the medium was removed, and the cells were washed six times using 1× PBS. All the specimens were photographed using an Olympus FV1000-IX81 confocal laser scanning microscope. Confocal images of HeLa cells stained with **TPE-DNS** and **TPE-NBD** taken under continuous excitation at 405 nm. The fluorescence images of green channel were collected at 450–550 nm and 500–570 nm, respectively.

Computational Details

Density functional theory (DFT) and time dependent (TD)-DFT calculations were performed using *Gaussian 09*.^{S4} These calculations employ the B3LYP functional, in combination with the 6-31+G(d,p) basis set. Solvent effects (acetonitrile) were taken into account using the IEFPCM model. Frequency checks were carried out after each geometry optimization to ensure that the minima on the potential energy surfaces were found.

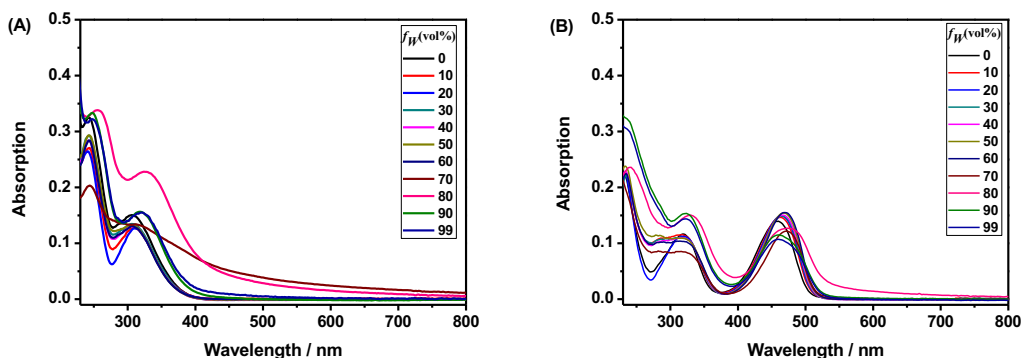


Fig. S1 UV—vis absorption spectra of **TPE-DNS** (A) and **TPE-NBD** (B) (10 μ M) in CH_3CN –water mixtures with different volume fractions of water (f_W).

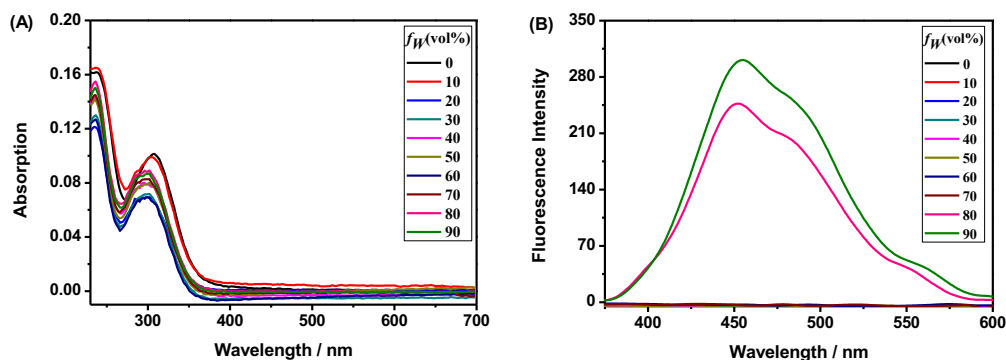


Fig. S2 Absorption (A) and fluorescent spectra (B) of **TPE** (10 μ M) in the binary mixture of acetonitrile–water with different volume fractions of water (f_W ; $\lambda_{\text{ex}} = 320$ nm).

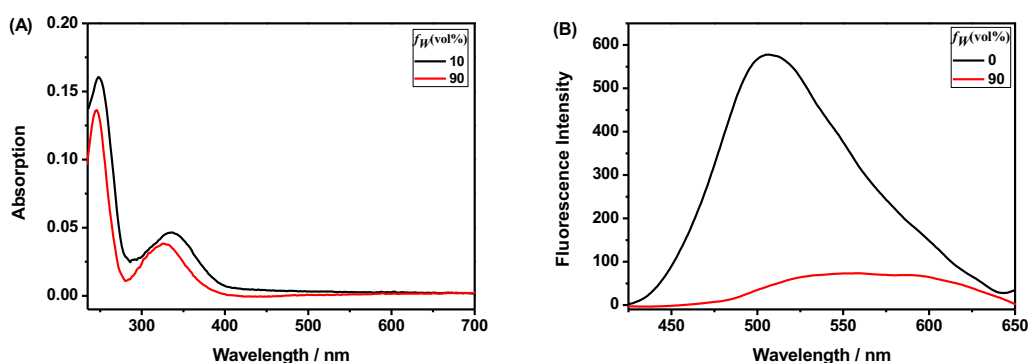


Fig. S3 Absorption (A) and fluorescent (B) spectra of **Bu-DNS** (10 μ M) in acetonitrile–water with different volume fractions of water (f_W ; $\lambda_{\text{ex}} = 340$ nm;).

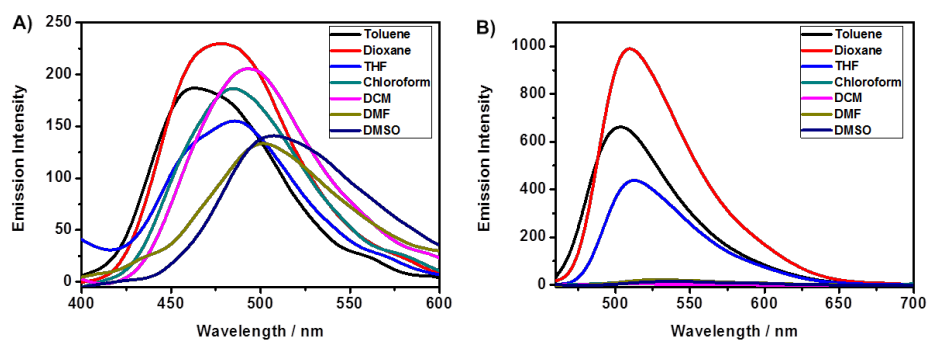


Fig. S4 Fluorescent spectra of (A) **TPE-DNS** (10 μ M; λ_{ex} = 340 nm; Slit: 15/2.5 nm) and (B) **TPE-NBD** (10 μ M; λ_{ex} = 450 nm; Slit: 15/2.5 nm) in various of solvents with different polarities.

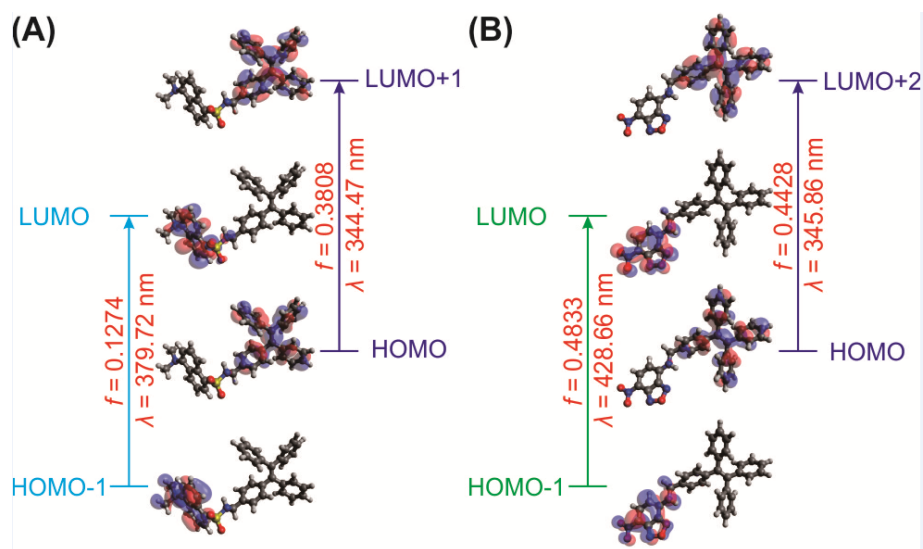


Fig. S5 Frontier molecular orbital profiles of **TPE-DNS** (A) and **TPE-NBD** (B) based on TDDFT (B3LYP/6-31G*) calculations.

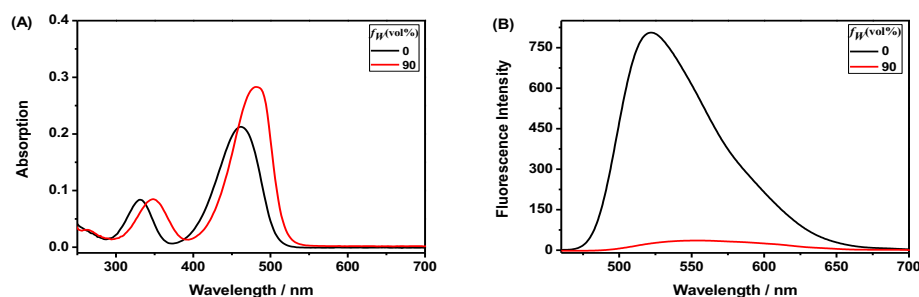
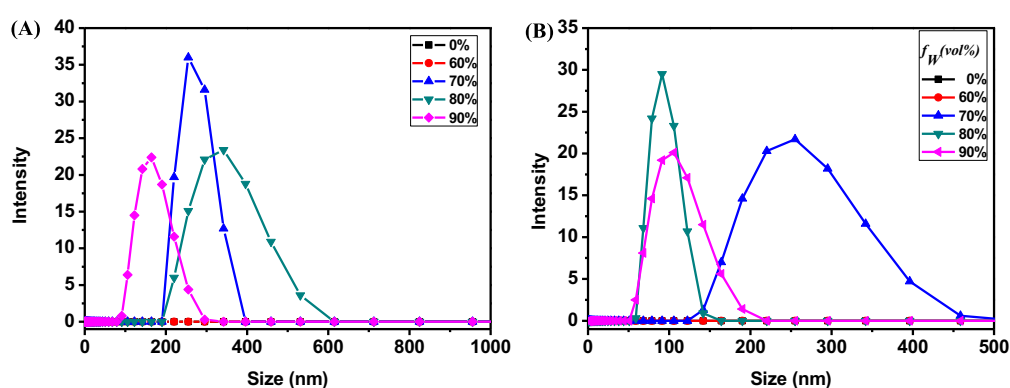
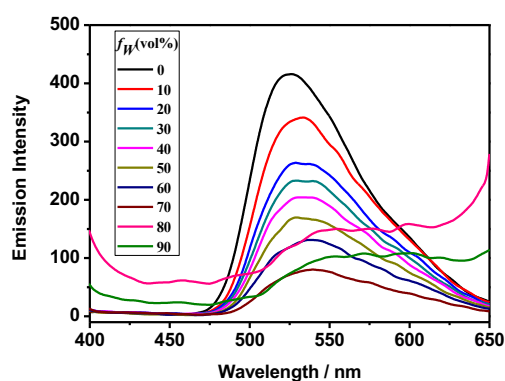


Fig. S6 Absorption (A) and fluorescent (B) spectra of **Bu-NBD** (10 μ M) in acetonitrile–water with different volume fractions of water (f_W ; λ_{ex} = 450 nm).

Table S1. Major electronic excitations for **TPE-NBD** and **TPE-DNS** in acetonitrile.

Compound	Excited state	λ/nm [eV]	Osc. str (f)	Major contributions
TPE-NBD	$S_0 \rightarrow S_2$	428.66 [2.8923]	0.4833	HOMO-1 $\rightarrow$ LUMO (95%)
	$S_0 \rightarrow S_7$	345.86 [3.5849]	0.4428	HOMO $\rightarrow$ LUMO+2 (97%)
TPE-DNS	$S_0 \rightarrow S_1$	379.72 [3.2651]	0.1274	HOMO-1 $\rightarrow$ LUMO (97%)
	$S_0 \rightarrow S_3$	344.47 [3.5992]	0.3808	HOMO $\rightarrow$ LUMO+1 (92%)

**Fig. S7** Particle size distributions of **TPE-DNS** (a) and **TPE-NBD** (b) aggregates in the binary mixture of acetonitrile and water, as a function of water fraction (f_w).**Fig. S8** Fluorescent spectra of **TPE-NBD** (10 μM) in the binary mixture of acetonitrile and water with different water fractions (f_w). (λ_{ex} = 340 nm; Slit: 10/10 nm)

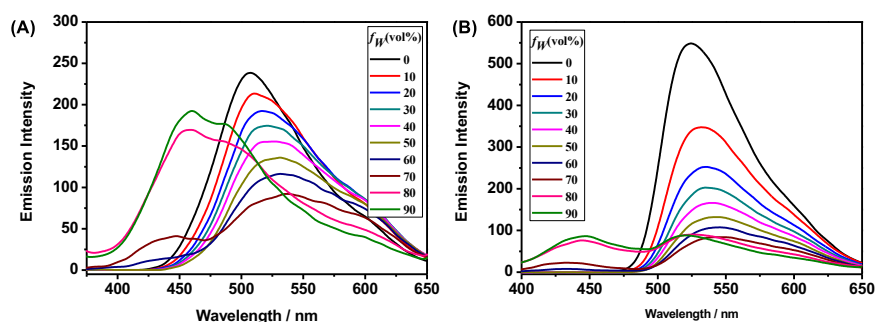


Fig. S9 Fluorescent spectra of the physical mixture of **TPE** (10 μM) with (a) **Bu-DNS** (10 μM) and (b) **Bu-NBD** (10 μM), as a function of water fraction (f_W) in the binary mixture of acetonitrile and water ($\lambda_{\text{ex}} = 340$ nm; Slit: 15/3 nm).

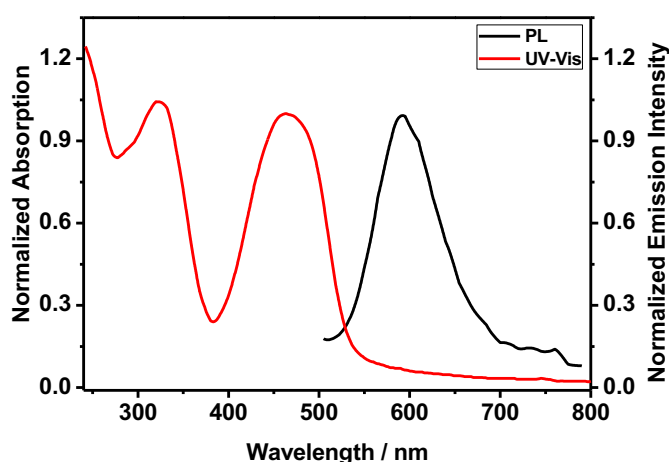


Fig. S10 Normalized solid-state absorption and emission spectra of **TPE-NBD**. The emission spectrum was collected at an excitation wavelength of 450 nm (slit: 10/5 nm)

Table S2. Crystal data and structure refinement parameters of **TPE-DNS**.

Compound	TPE-DNS
Empirical formula	$\text{C}_{39}\text{H}_{34}\text{N}_2\text{O}_2\text{S}$
Formula weight	594.74
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	Cc
a (Å)	9.805(5) Å

b (Å)	39.34(2) Å
c (Å)	9.106(5) Å
α (deg)	90
β (deg)	115.444(8)
γ (deg)	90
Volume(Å ³)	3172(3)
Z	4
Density (calculated)	1.245 Mg/m ³
Absorption coefficient	0.139 mm ⁻¹
F(000)	1256
Crystal size	0.12 × 0.10 × 0.08 mm ³
Theta range for data collection	2.071 to 27.429°.
Index ranges	-12 ≤ h ≤ 12, -50 ≤ k ≤ 46, -11 ≤ l ≤ 11
Reflections collected	13289
Independent reflections	6880 [R(int) = 0.0462]
Completeness to theta = 26.00	99.8 %
Absorption correction	None
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	6880 / 83 / 454
Goodness-of-fit on F ²	0.998
Final R indices [I > 2σ(I)]	R1 = 0.0608, wR2 = 0.1325
R indices (all data)	R1 = 0.1189, wR2 = 0.1594
Largest diff. peak and hole	0.242 and -0.187 e.Å ⁻³

Table S3. Crystal data and structure refinement parameters of **TPE-NBD**.

Compound	TPE-NBD
Empirical formula	C ₃₃ H ₂₄ N ₄ O ₃
Formula weight	524.56
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic

Space group	P2(1)/c
a (Å)	22.854(3) Å
b (Å)	9.0152(13) Å
c (Å)	13.197(2) Å
α (deg)	90
β (deg)	96.266(3)
γ (deg)	90
Volume(Å ³)	2702.8(7)
Z	4
Density (calculated)	1.289 Mg/m ³
Absorption coefficient	0.084 mm ⁻¹
F(000)	1096
Crystal size	0.2 × 0.2 × 0.15 mm ³
Theta range for data collection	0.90 to 29.00°.
Index ranges	-31 ≤ h ≤ 30, -11 ≤ k ≤ 12, -18 ≤ l ≤ 18
Reflections collected	25150
Independent reflections	7168 [R(int) = 0.0505]
Completeness to theta = 26.00	99.7 %
Absorption correction	None
Refinement method	Full-matrix least-squares on F ²
Data / restraints / parameters	7168 / 0 / 361
Goodness-of-fit on F ²	1.038
Final R indices [I > 2σ(I)]	R1 = 0.0476, wR2 = 0.1157
R indices (all data)	R1 = 0.1070, wR2 = 0.1533
Largest diff. peak and hole	0.223 and -0.246 e.Å ⁻³

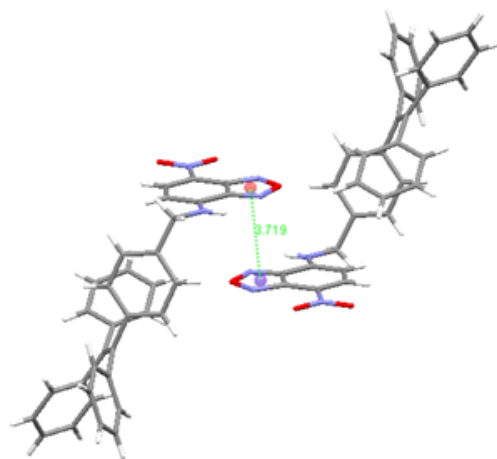


Fig. S11 Weak π - π stacking interactions between two layers of **NBD** moieties in the crystal structure of **TPE-NBD**.

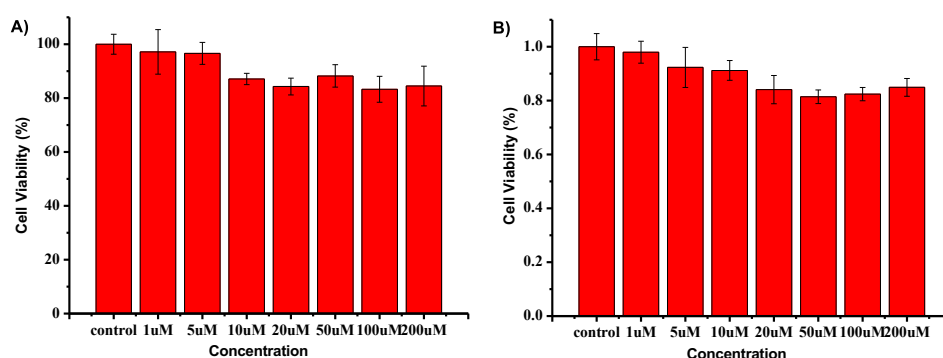


Fig. S12 Cell viabilities of HeLa cells stained with **TPE-DNS** and **TPE-NBD** at various concentrations.

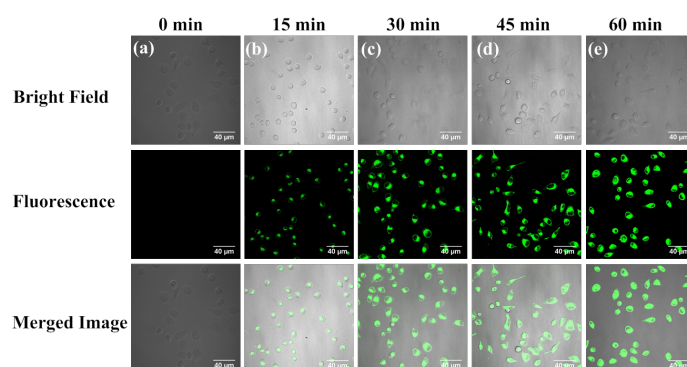


Fig. S13 Confocal microscope images of HeLa cells in the presence of **TPE-NBD** (5 μ M): (a) the cells incubated with **TPE-NBD** for 0 min; (b) the cells incubated with **TPE-NBD** for 15 min; (c) the cells incubated with **TPE-NBD** for 30 min; (d) the cells incubated with **TPE-NBD** for 45 min; (e) the cells incubated with **TPE-NBD** for 60 min.

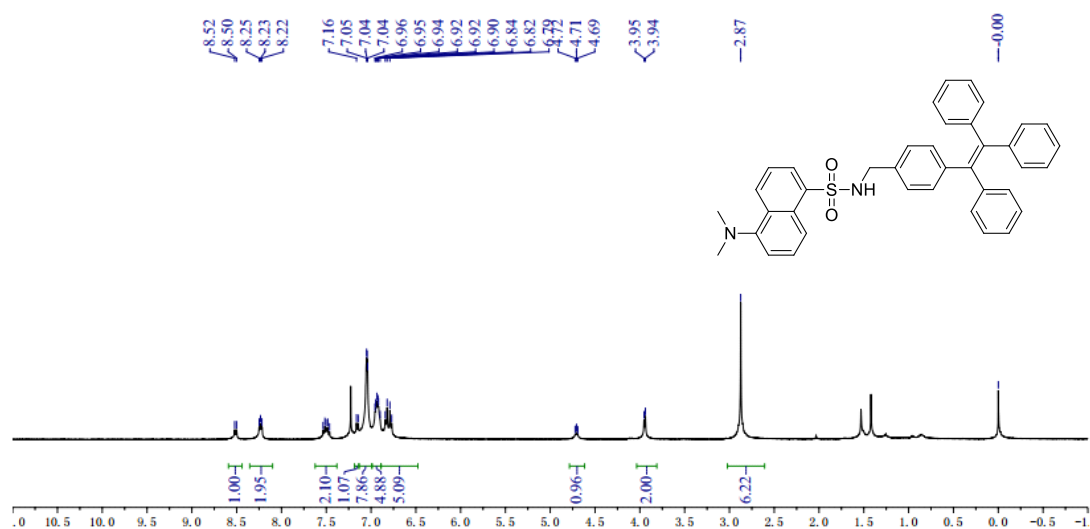


Fig. S14 ¹H NMR spectrum of TPE-DNS

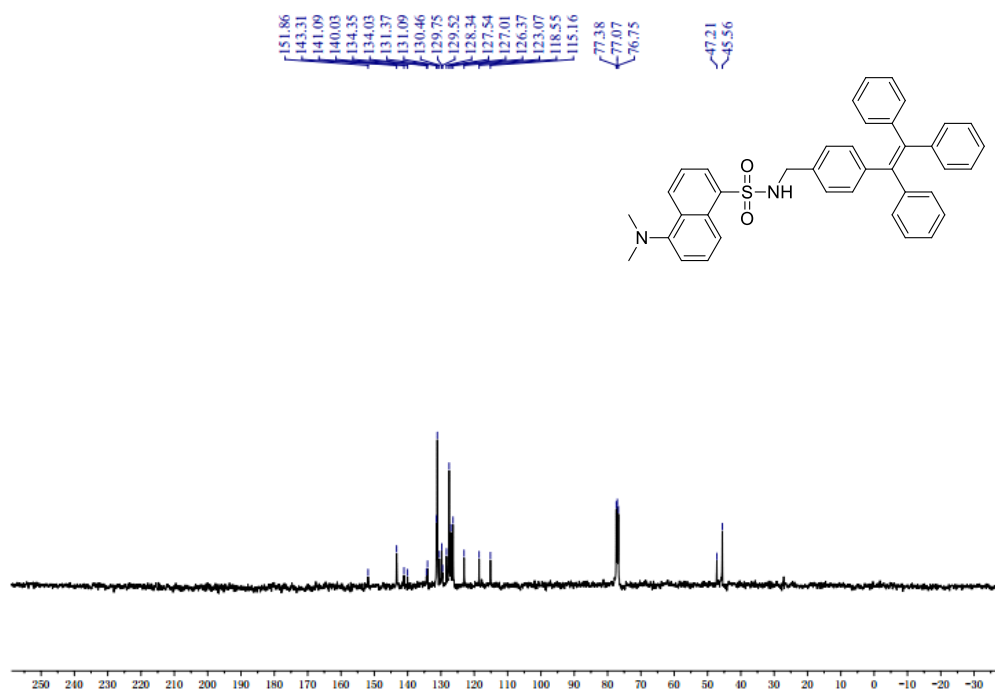


Fig. S15 ¹³C NMR spectrum of TPE-DNS

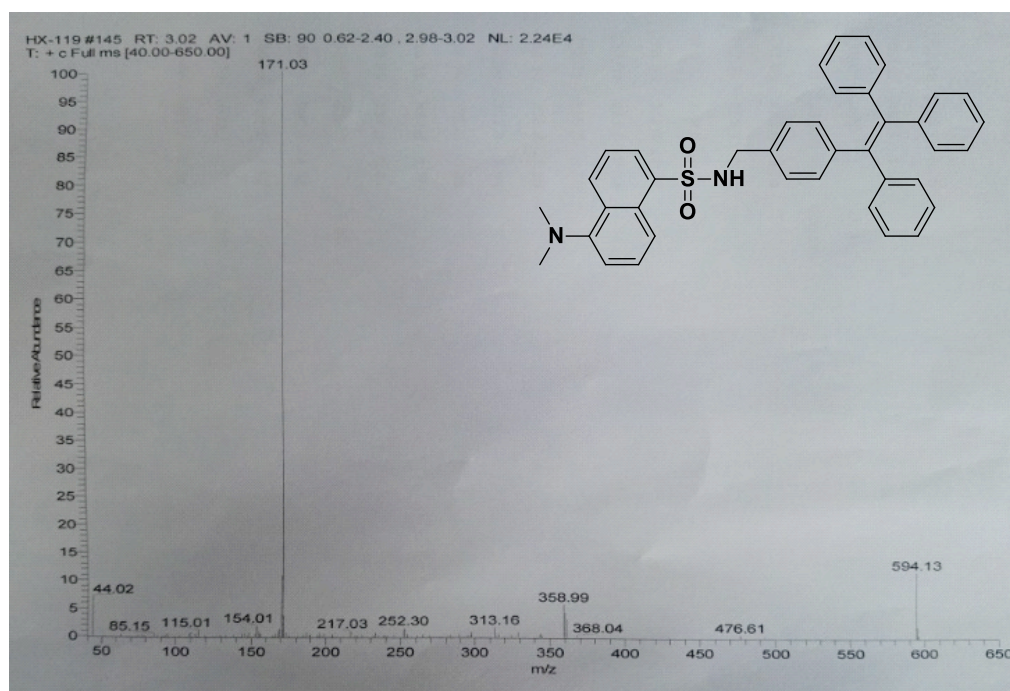


Fig. S16 EI mass spectrum of TPE-DNS

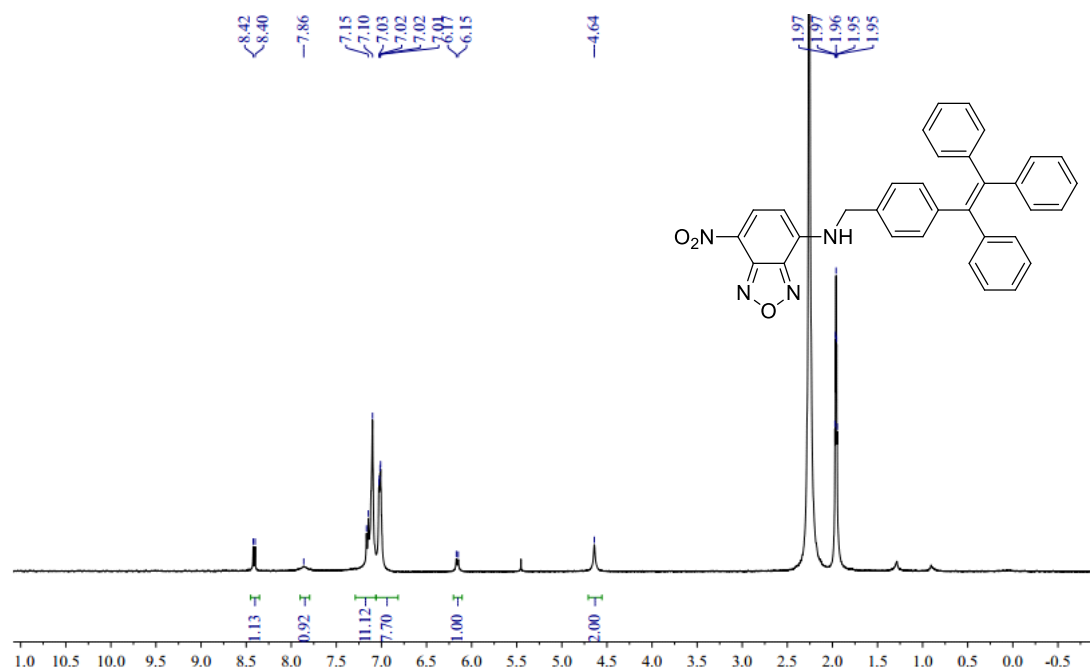


Fig. S17 ¹H NMR spectrum of TPE-DNS

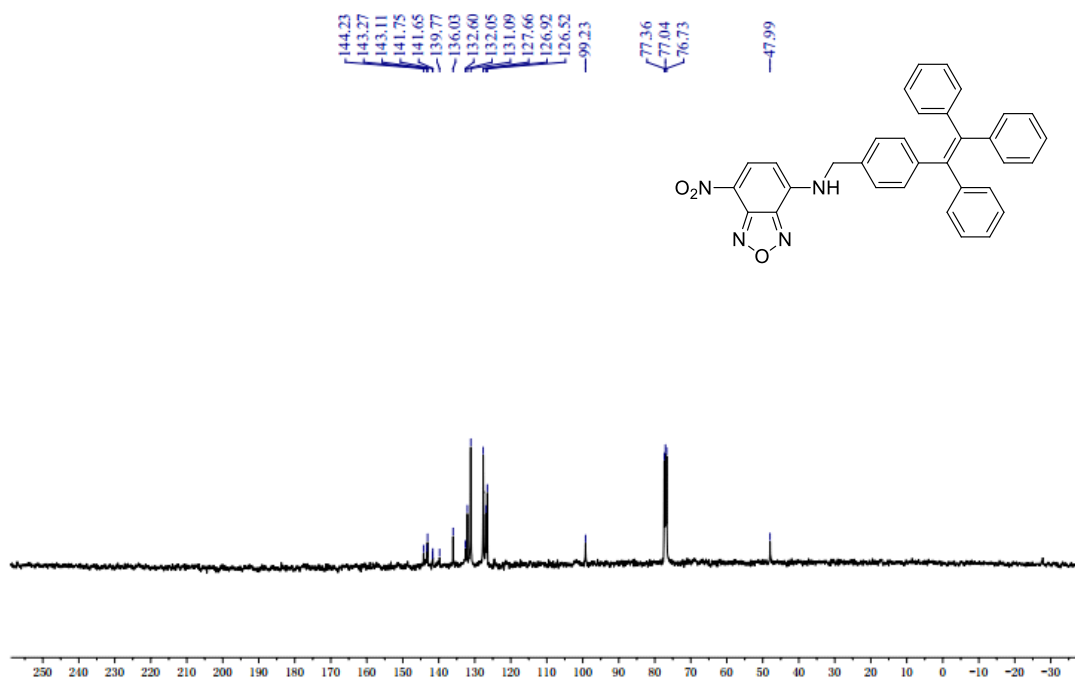


Fig. S18 ¹³C NMR spectrum of TPE-DNS

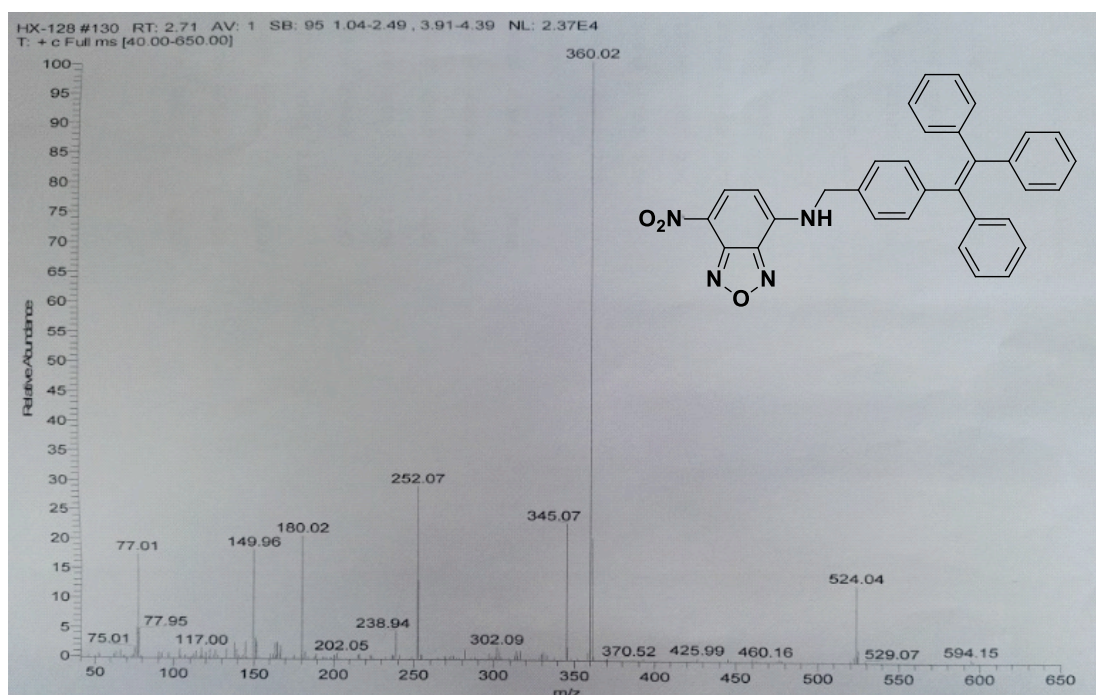


Fig. S19 EI mass spectrum of TPE-DNS

Reference

- S1 (a) Y. Yang, A. J. Mijalis, H. Fu, C. Agosto, K. J. Tan, J. D. Batteas and D. E. Bergbreiter, *J. Am. Chem. Soc.*, 2012, **134**, 7378. (b) W. Chen, H. Luo, X. Liu, J. W. Foley and X. Song, *Anal. Chem.*, 2016, **88**, 3638.
- S2 Sheldrick GM. SHELXS-97, a program for crystal structure solution. Germany: Göttingen; 1997.
- S3 Sheldrick GM. SHELXL-97, a program for crystal structure refinement. Germany: Göttingen; 1997.
- S4 M. J. Frisch, G. W. Trucks, H. B. Schlegel, et al., Gaussian09, Revision A.01. Gaussian, Gaussian, Inc., Wallingford, Conn, USA, 2009.