Electronic Supplementary Information for

Bright Near-Infrared α-TetraphenyletheneBODIPY Nanoprobes with High Aggregated State Emission Quantum Yields in Aqueous System for Lipid Droplet-Specific Imaging

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Contents

1. Crystal packing and selected parameters

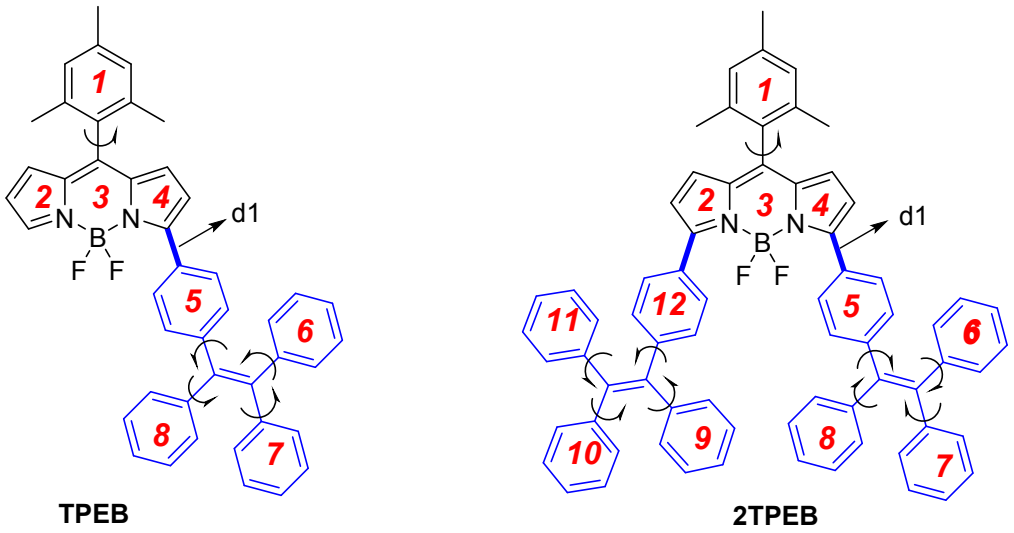


Table S1. Selected bond lengths [Å] and dihedral angles [deg] of **TPEB** and **2TPEB** obtained from X-ray crystallography.

	TPEB	**2TPEB**
B-N bond length (Å)	1.566 (1) 1.55 (3)	1.559 (5) 1.569 (5)
dihedral angles between *meso*-mesityl group and dipyyrin core (deg)	86.76 (5)	83.99 (2)
dihedral angles of two pyrrole rings in dipyyrin core (deg)	5.48 (7)	9.04 (2)
Newly formed C-C single bond **d1** length (Å)	1.468 (2)	1.466 (5)

Table S2. Crystal data collection and structure refinement details for **TPEB** and **2TPEB** obtained from crystallography.

Compound	**TPEB**	**2TPEB**	**Compound**	**TPEB**	**2TPEB**
Formula	$C_{44}H_{35}BF_2N_2$	$C_{70}H_{53}BF_2N_2$	Z'	1	1
$D_{calc.}$/g cm^{-3}	1.223	1.183	Wavelength/Å	0.710760	0.710760
μ/mm^{-1}	0.078	0.072	Radiation type	MoK$\square$	MoK$\square$
Formula Weight	640.55	970.95	$\Theta_{\mu\nu}$/°	2.859	2.866
Colour	red	red	$\Theta_{\mu\alpha\xi}$/°	27.552	28.053
Shape	plate	plate	Measured Refl.	149655	167188
Size/mm^3	0.22×0.21×0.20	0.22×0.21×0.20	Independent Refl.	7999	12582
T/K	298.15	298.15	Reflections with I > 2(I)	6199	6068
Crystal System	monoclinic	monoclinic	R_{int}	0.0395	0.1437
Space Group	$C2/c$	$C2/c$	Parameters	445	679
a/Å	28.5519(9)	21.990(4)	Restraints	0	0
b/Å	10.6876(4)	17.086(3)	Largest Peak	0.198	0.261
c/Å	25.3317(10)	30.822(7)	Deepest Hole	-0.198	-0.330
α/°	90	90	GooF	1.036	1.039
β/°	115.8420(10)	109.653(8)	wR_2 (all data)	0.1293	0.3004
γ/°	90	90	wR_2	0.1138	0.2379
V/Å^3	6957.0(4)	10906(4)	R_1 (all data)	0.0676	0.1991
Z	8	8	R_1	0.0487	0.1056

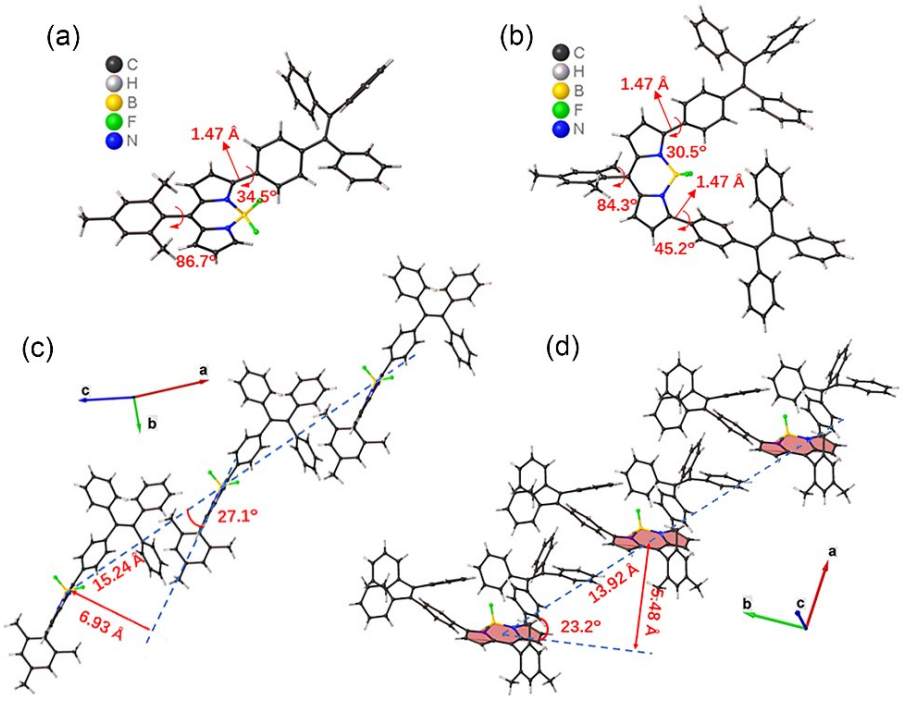
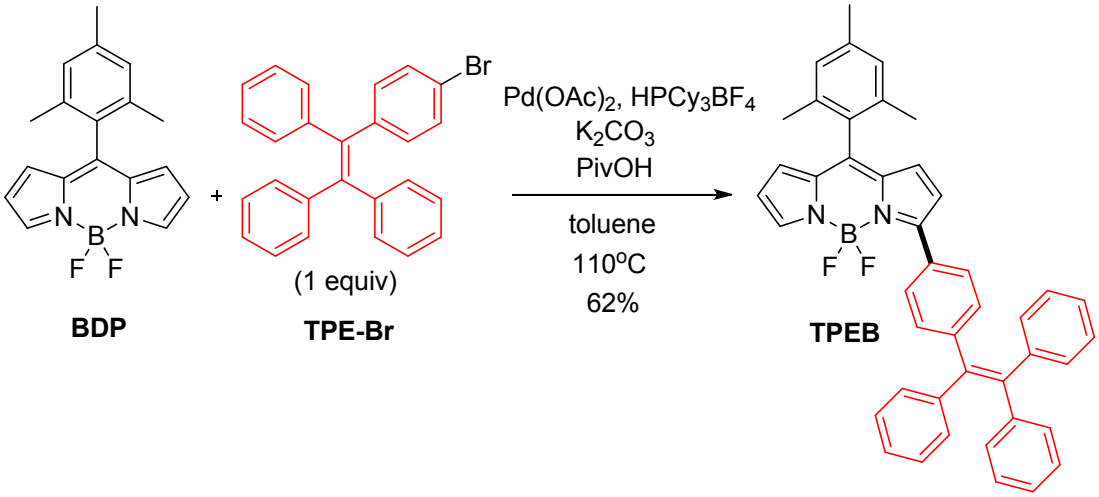


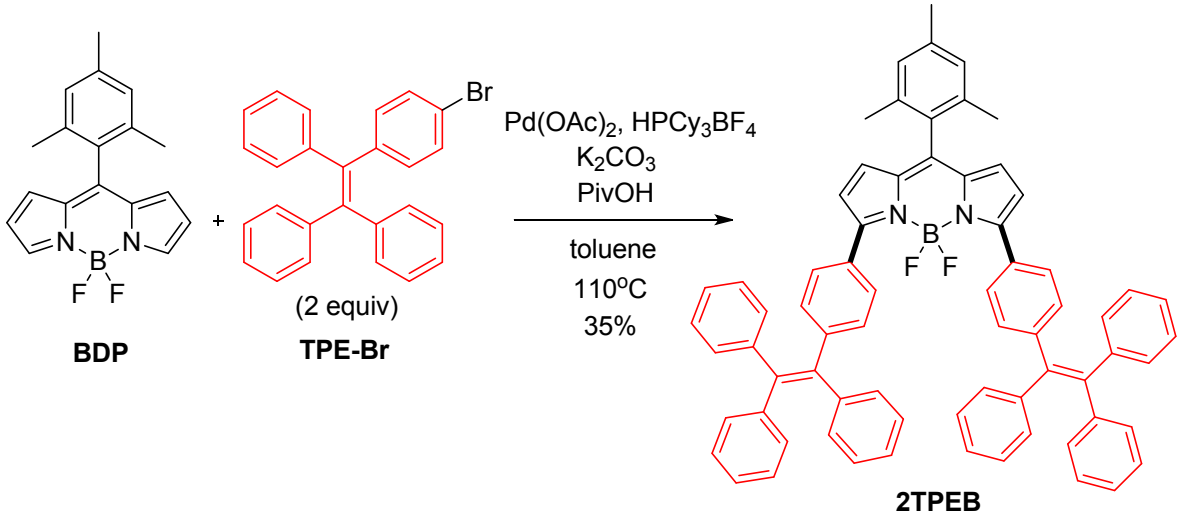
Fig. S1. (a, b) X-ray crystal structures of **TPEB** and **2TPEB**; (c, d) Crystal-packing pattern of **TPEB** and **2TPEB** between the adjacent interlayered crystals. Interlayer distances of **TPEB** is 6.93 Å; slip angles of **TPEB** is 27.1°; Interlayer distance of **2TPEB** is 5.48 Å and slip angle is 23.2°.

2. Synthetic routes for TPEB and 2TPEB

BDP was synthesized by following the procedures according to the literature (C. Yu, L. Jiao, H. Yin, J. Zhou, W. Pang, Y. Wu, Z. Wang, G. Yang and E. Hao, *Eur. J. Org. Chem.*, 2011, **28**, 5460.).



Scheme S1. Synthetic route and yields of **TPEB** *via* palladium-catalyzed C-H arylation.



Scheme S2. Synthetic route and yields of **2TPEB** *via* palladium-catalyzed C-H arylation.

3. Photophysical properties

Table S3. Photophysical properties of **TPEB** and **2TPEB** in solutions and solid state at room temperature.

dyes	solvent	λ_{abs}^{max} [nm]	$\varepsilon_{abs}^{max\ a}$	λ_{em}^{max} [nm]	Φ^{b}	Stokes shift[c] [cm^{-1}]	τ^{d} [ns]
TPEB	hexane	547	72100	580	0.66	1040	5.21
	toluene	553	65800	591	0.65	1160	4.41
	$CHCl_3$	551	62200	597	0.58	1400	4.42
	CH_2Cl_2	547	62700	601	0.35	1640	3.17
	THF	550	69500	593	0.54	1320	4.39
	CH_3OH	543	63100	591	0.02	1500	2.12
	CH_3CN	541	59400	596	0.01	1710	5.25
	water[e]	554	35800	684	0.25	3430	-
	water[f]	554	35800	660	0.35	2900	-
	solid	-	-	700	0.10	-	-
2TPEB	hexane	592	63700	641	0.78	1290	5.77
	toluene	599	56000	649	0.96	1290	5.06
	$CHCl_3$	599	58700	650	0.93	1310	5.51
	CH_2Cl_2	596	58100	649	0.93	1370	5.39
	THF	600	62100	649	0.91	1260	5.47
	CH_3OH	587	63400	647	0.72	1580	5.06
	CH_3CN	584	54300	646	0.60	1640	4.34
	water[e]	612	38800	717	0.23	2730	-
	water[f]	598	38800	661	0.81	1590	-
	solid	-	-	790	0.22	-	-

[a]Data corresponding to the strongest absorption maximum, the unit for ε is $M^{-1}c^{-1}$. [b]Fluorescence quantum yields of **TPEB** were calculated using Rhodamine B (Φ = 0.49 in ethanol) as the reference. Fluorescence quantum yields of **2TPEB** were calculated using Cresyl Violet perchlorate (Φ = 0.54 in methanol) as the reference. [c]The Stokes shifts values are rounded to the nearest 10 cm^{-1}. [d]τ = fluorescence lifetimes. [e]Nanoparticles dispersed in deionized water containing 4% acetonitrile. [f]Nanoparticles wrapped with F127 dispersed in deionized water.

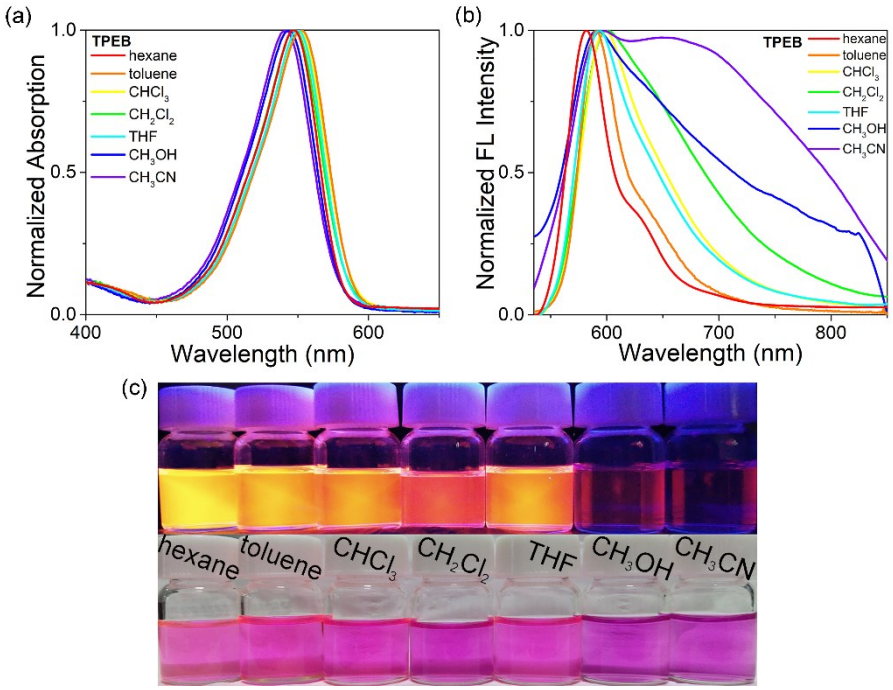


Fig. S2. Normalized absorption (a) and fluorescence spectra (b) of **TPEB** in different solutions, excited at 520 nm; (c) Photographs of **TPEB** in different solution under 365 nm UV lamp irradiation and daylight.

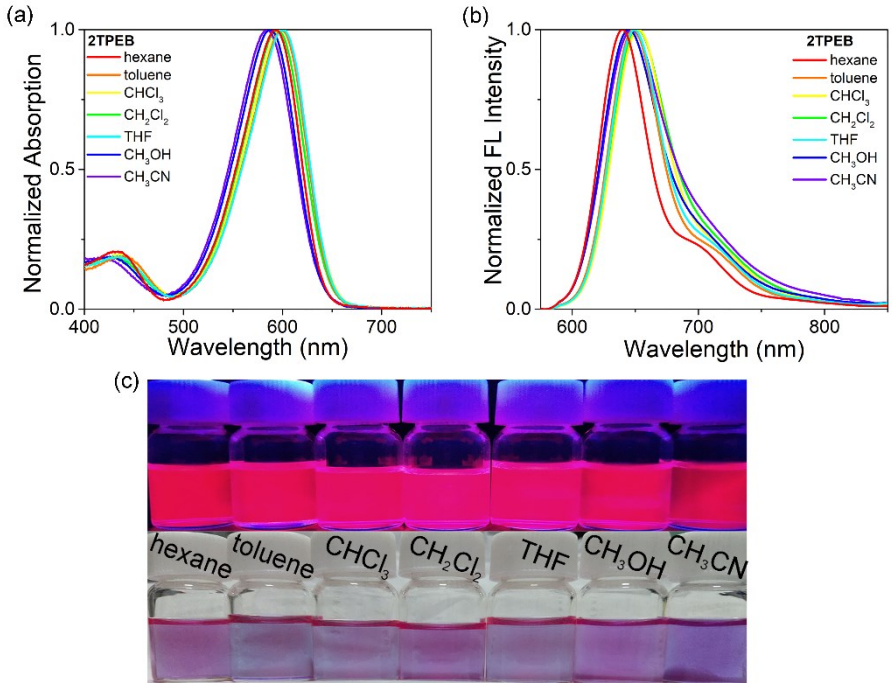


Fig. S3. Normalized absorption (a) and fluorescence spectra (b) of **2TPEB** in different solutions, excited at 520 nm; (c) Photographs of **2TPEB** in different solution under 365 nm UV lamp irradiation and daylight.

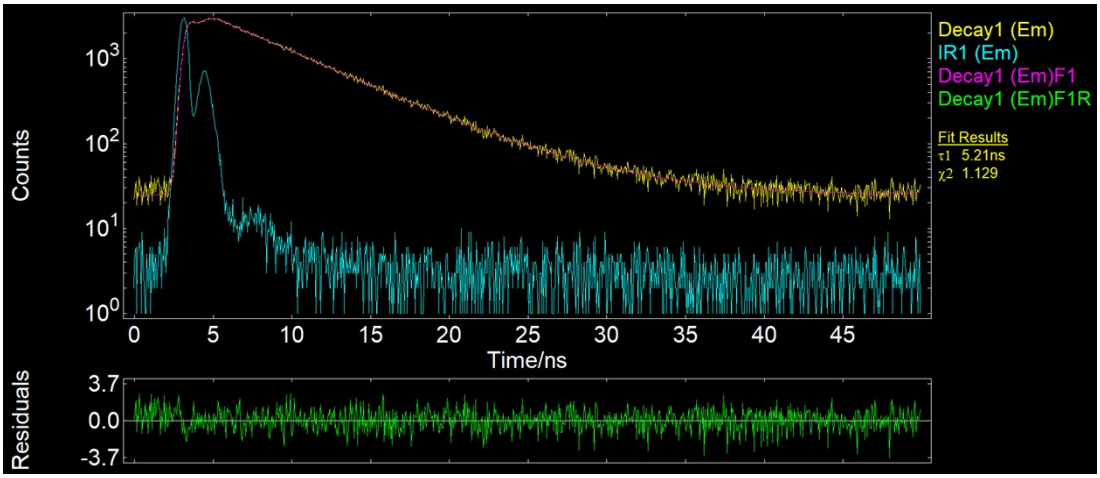


Fig. S4. Fluorescence lifetime of **TPEB** in n-hexane.

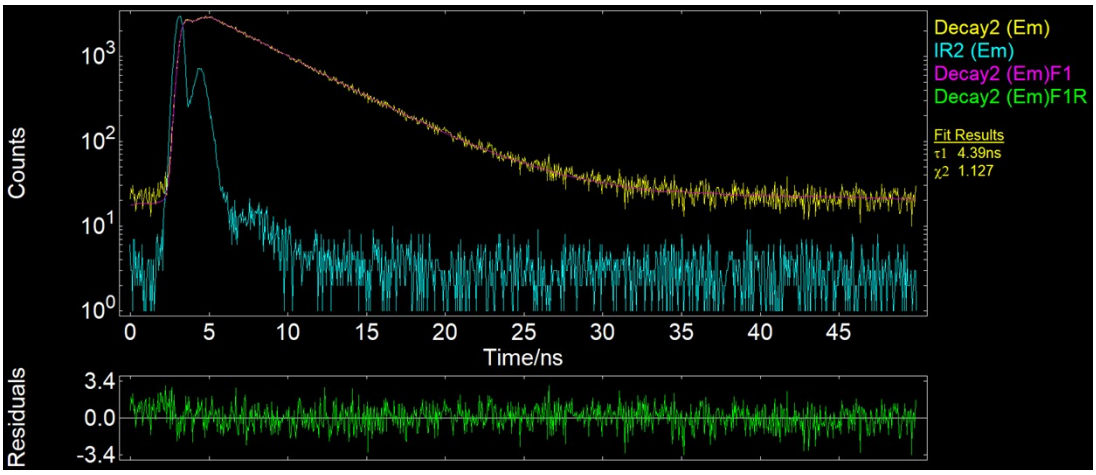


Fig. S5. Fluorescence lifetime of **TPEB** in tetrahydrofuran.

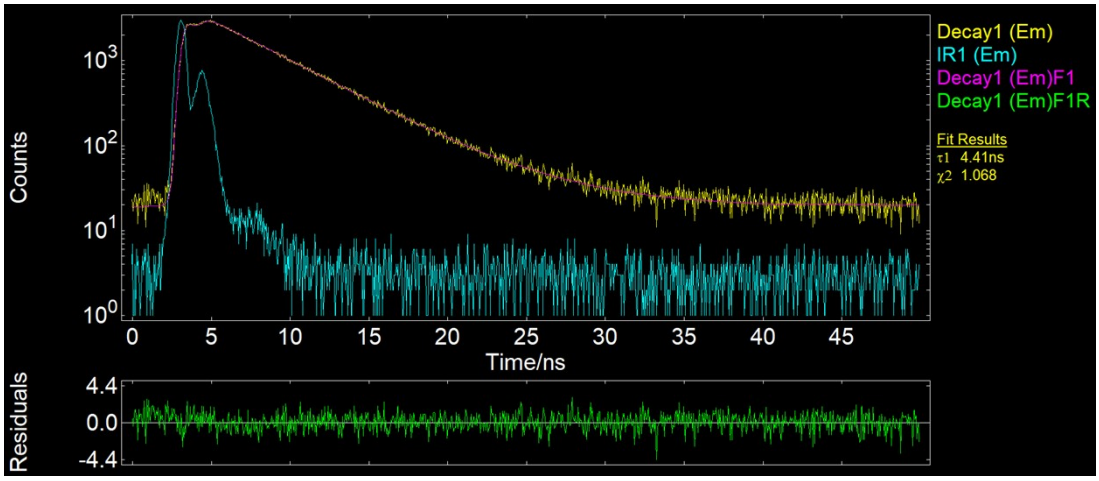


Fig. S6. Fluorescence lifetime of **TPEB** in toluene.

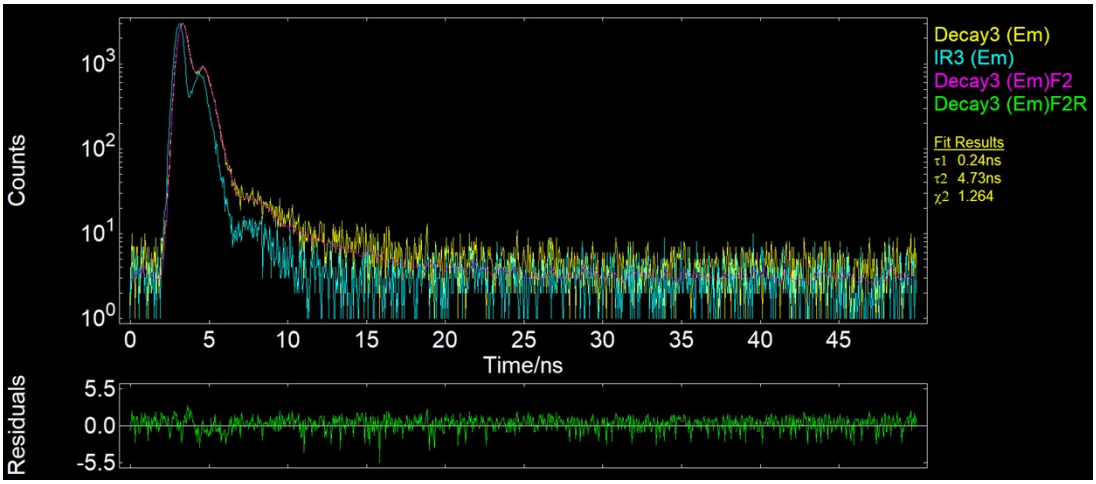


Fig. S7. Fluorescence lifetime of **TPEB** in methanol.

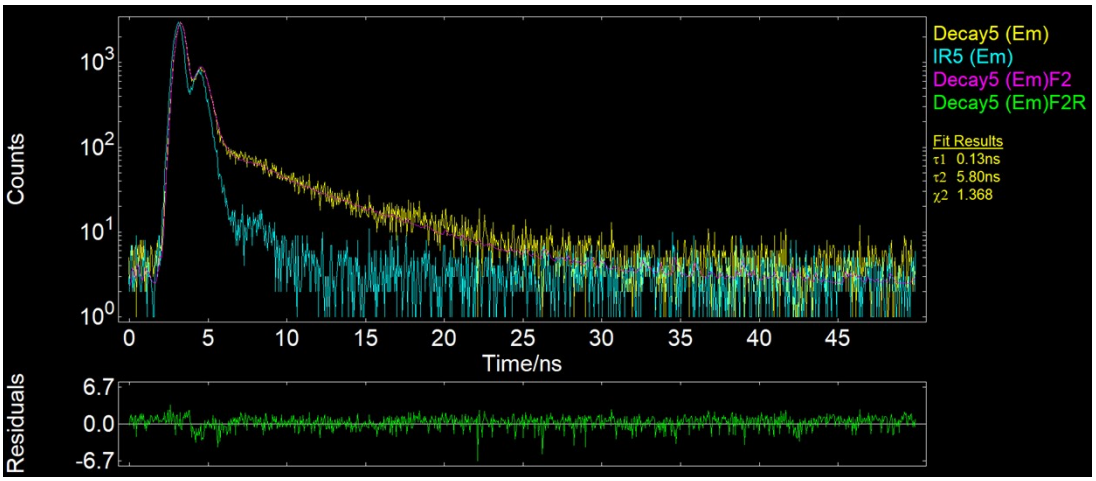


Fig. S8. Fluorescence lifetime of **TPEB** in acetonitrile.

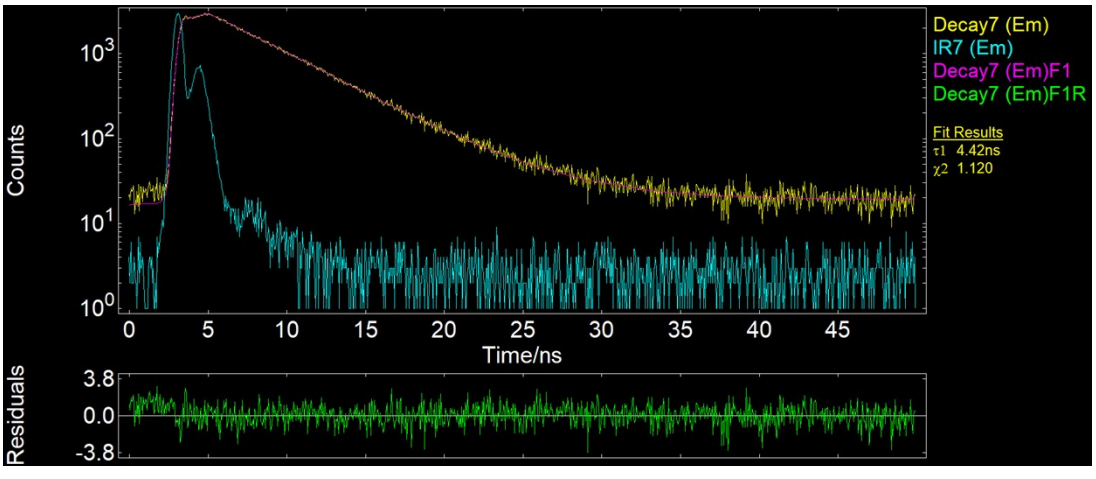


Fig. S9. Fluorescence lifetime of **TPEB** in chloroform.

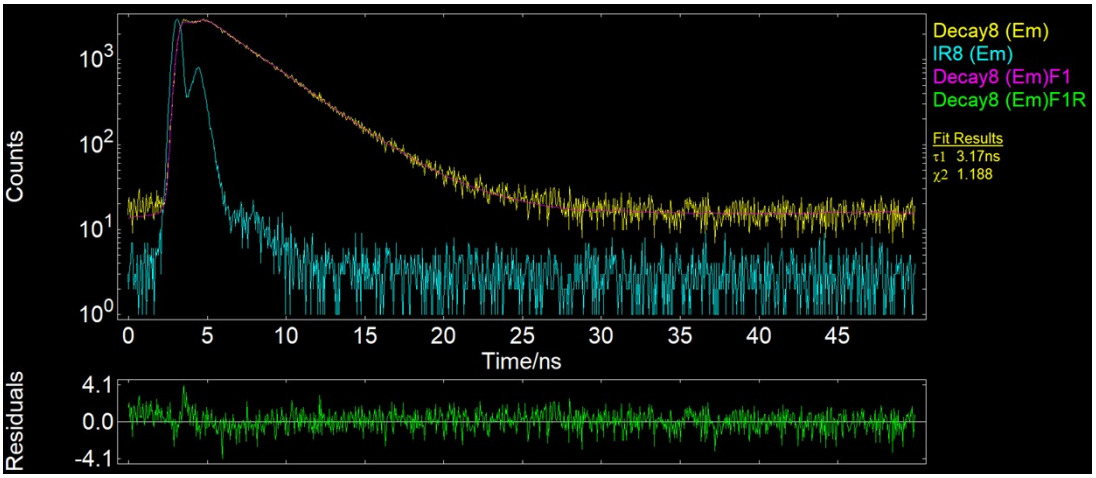


Fig. S10. Fluorescence lifetime of **TPEB** in dichloromethane.

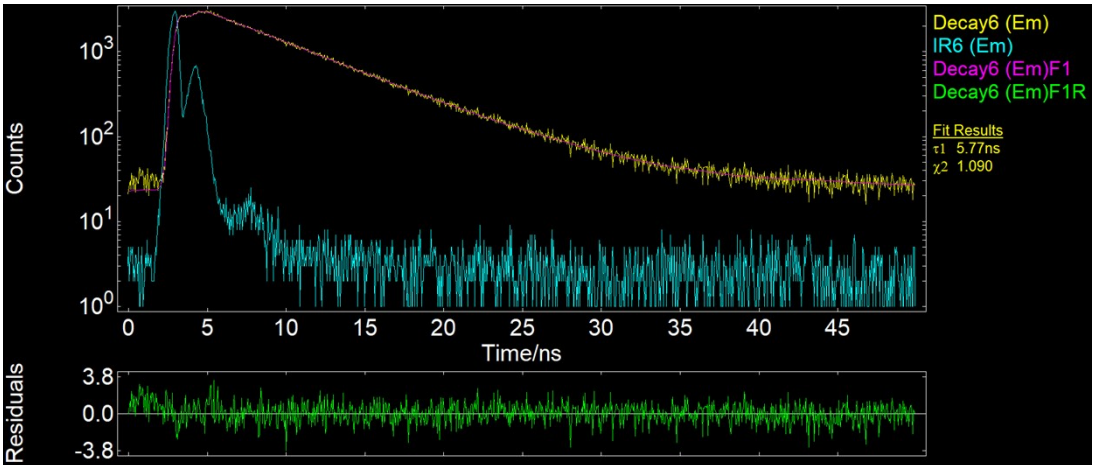


Fig. S11. Fluorescence lifetime of **2TPEB** in n-hexane.

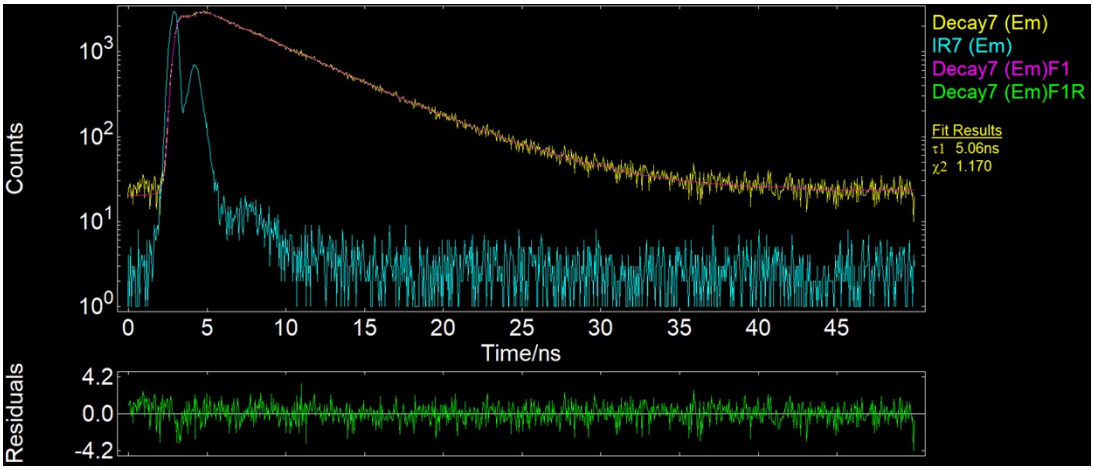


Fig. S12. Fluorescence lifetime of **2TPEB** in toluene.

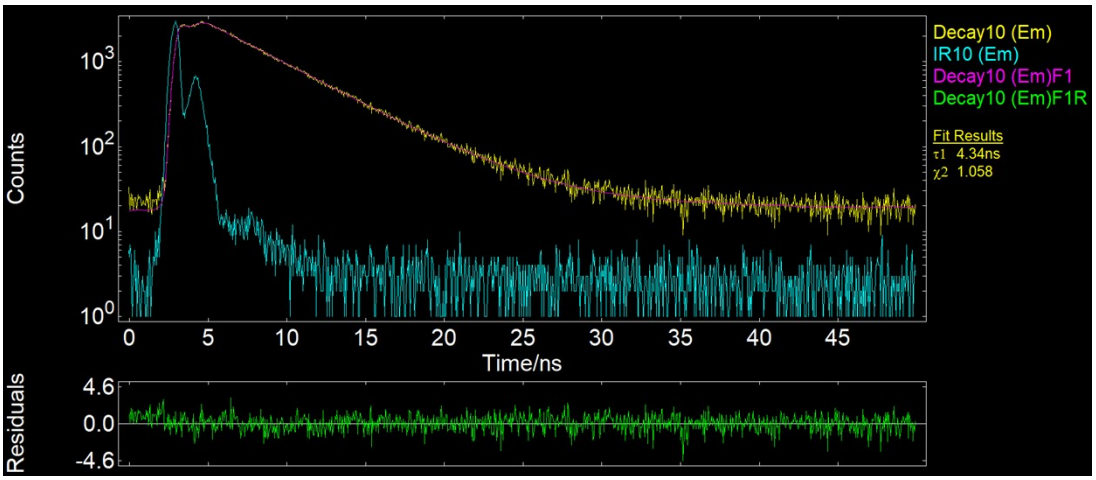


Fig. S13. Fluorescence lifetime of **2TPEB** in acetonitrile.

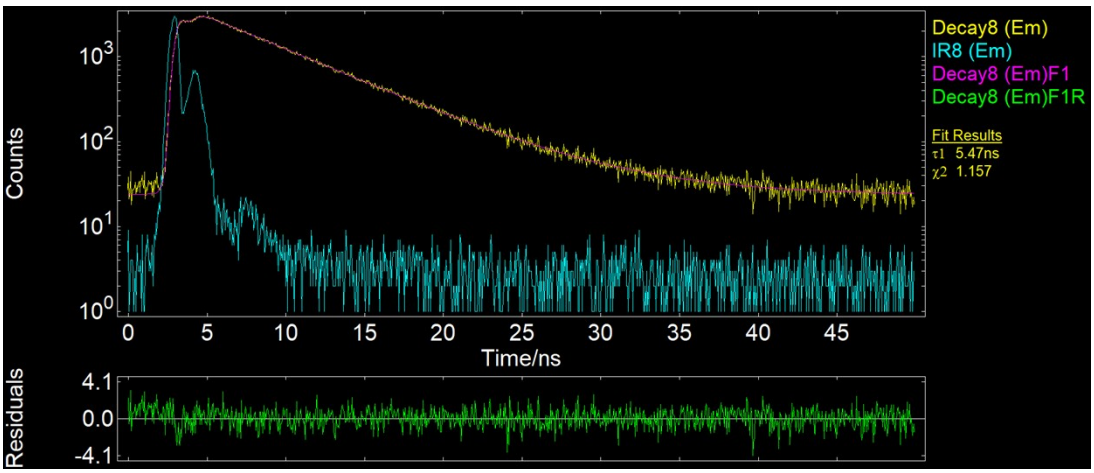


Fig. S14. Fluorescence lifetime of **2TPEB** in tetrahydrofuran.

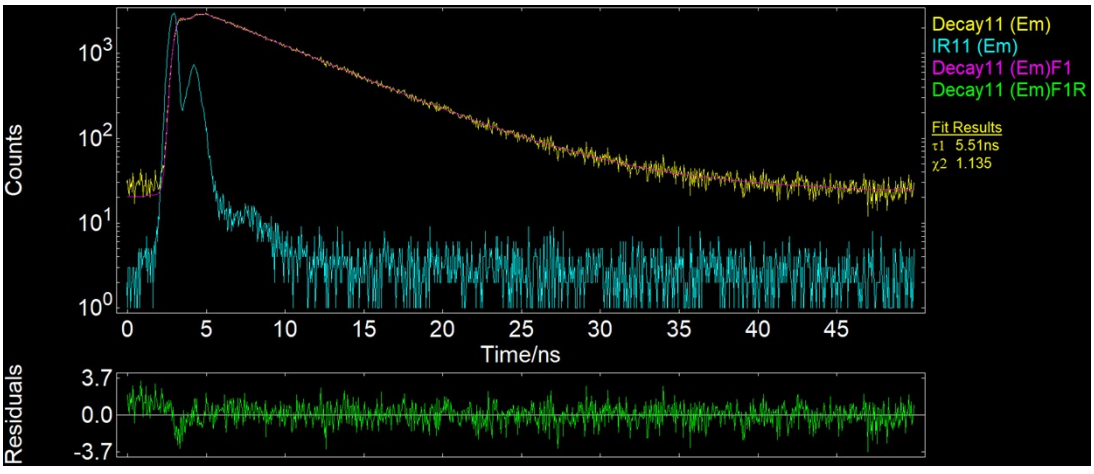


Fig. S15. Fluorescence lifetime of **2TPEB** in chloroform.

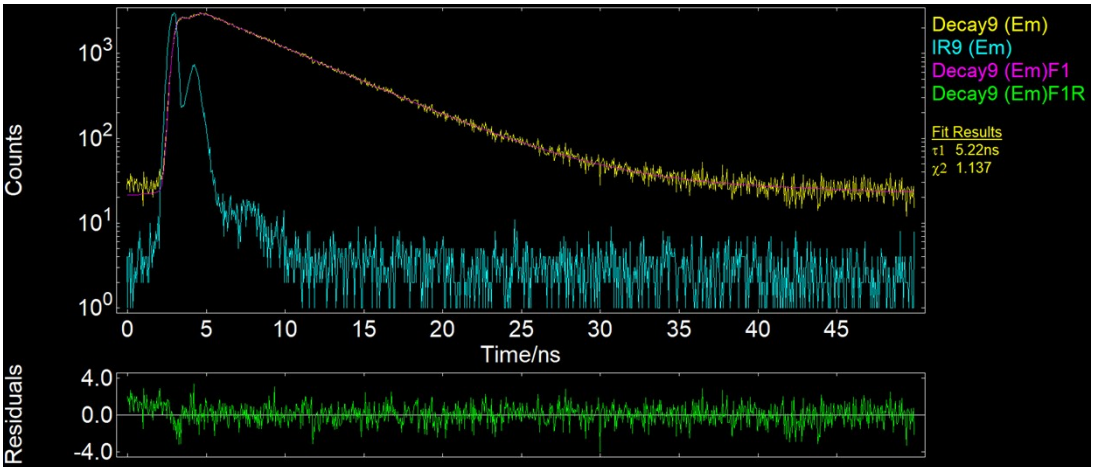


Fig. S16. Fluorescence lifetime of **2TPEB** in methanol.

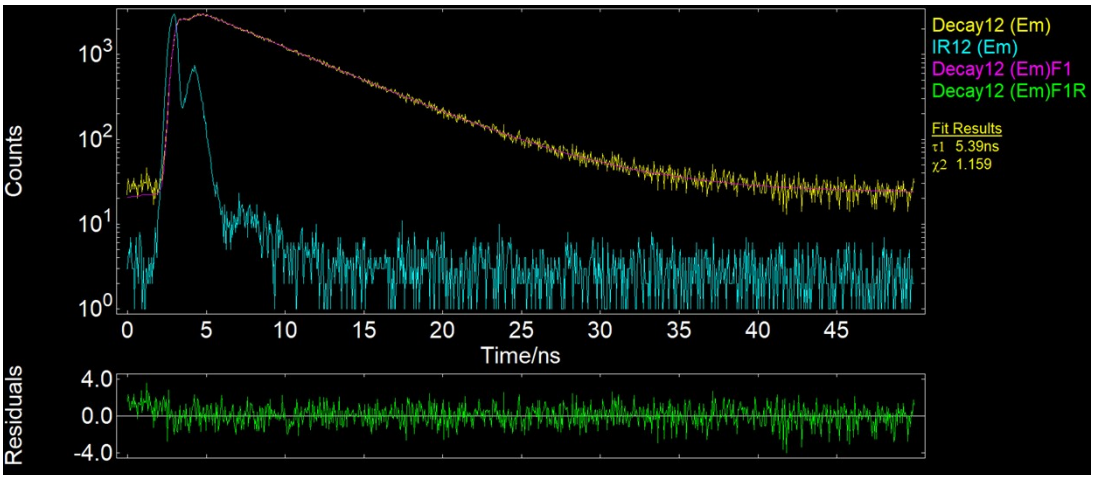


Fig. S17. Fluorescence lifetime of **2TPEB** in dichloromethane.

4. Aggregation studies of TPEB and 2TPEB

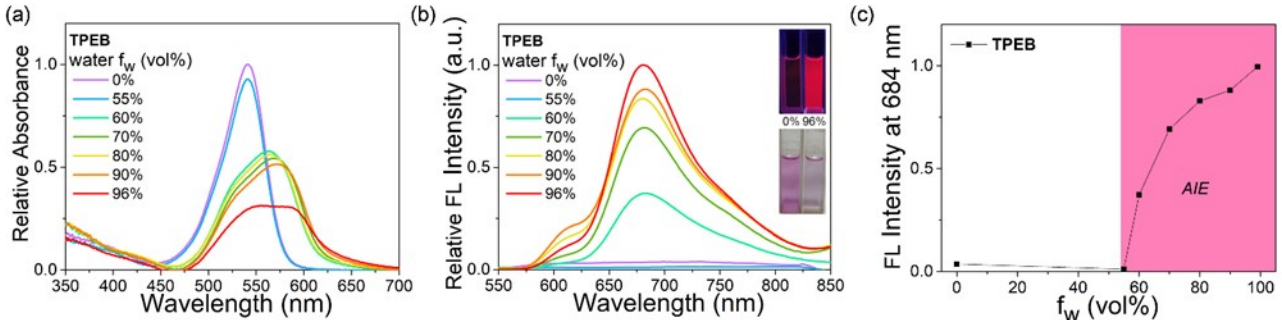


Fig. S18. Relative absorption spectra (a) and fluorescence spectra (b) of **TPEB** (10 μM) in acetonitrile/deionized water with different water fractions (*f*w), excited at 520 nm. (c) Plot of the fluorescent intensity versus different water fractions.

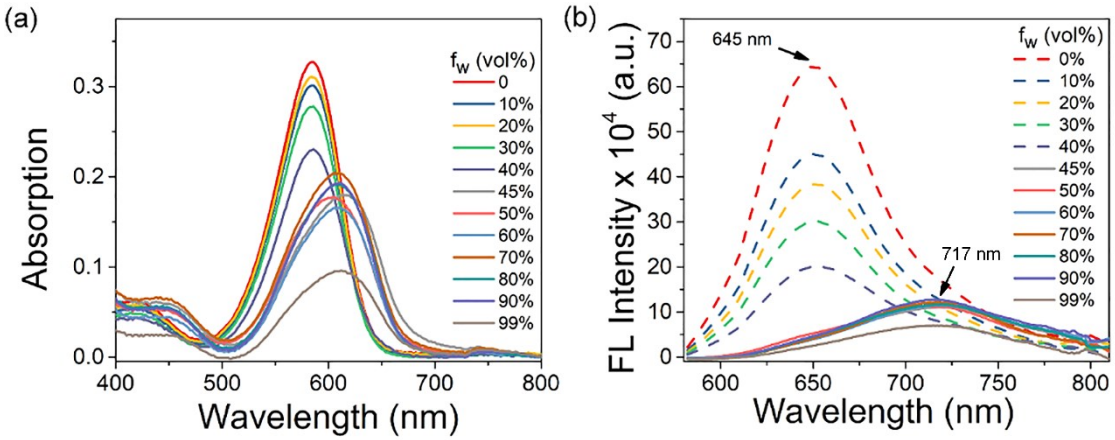


Fig. S19. (a) Absorption spectra of **2TPEB** in MeCN/water mixtures with di□erent water fractions (f_w). (b) Photoluminescence spectra of **2TPEB** in MeCN/water mixtures with di□erent water fractions (f_w). Solution concentration: 10 μM. Excitation wavelength: 550 nm.

5. Aggregation studies of TPEB or 2TPEB NPs wrapped with F127

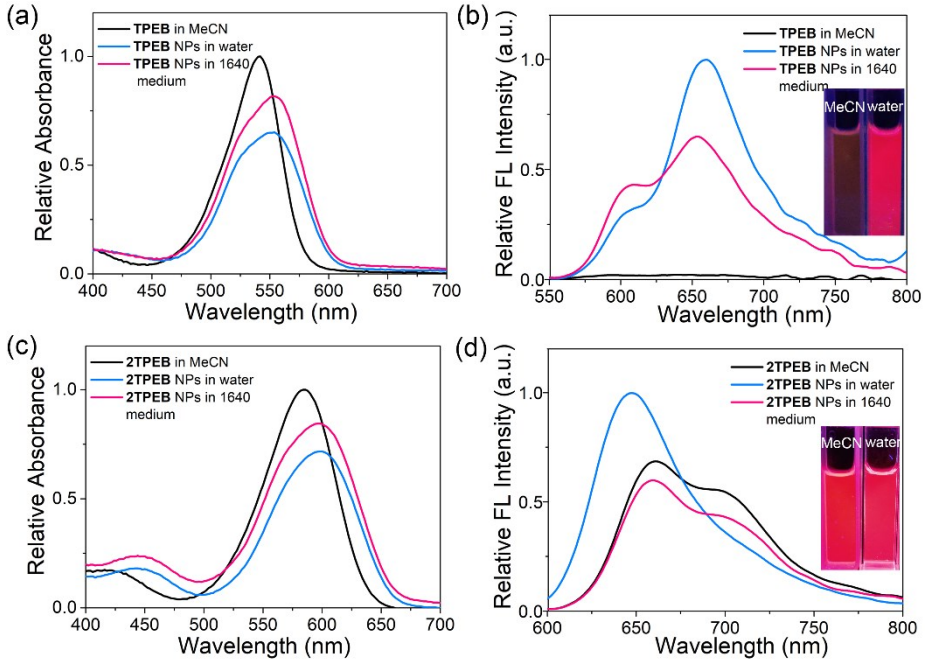


Fig. S20. Relative absorption (a, c) and (b, d) fluorescence spectra (in acetonitrile, deionized water and 1640 culture medium) of **TPEB** or **2TPEB** at 10 μM, excited at 520 nm for **TPEB** and 580 nm for **2TPEB**.

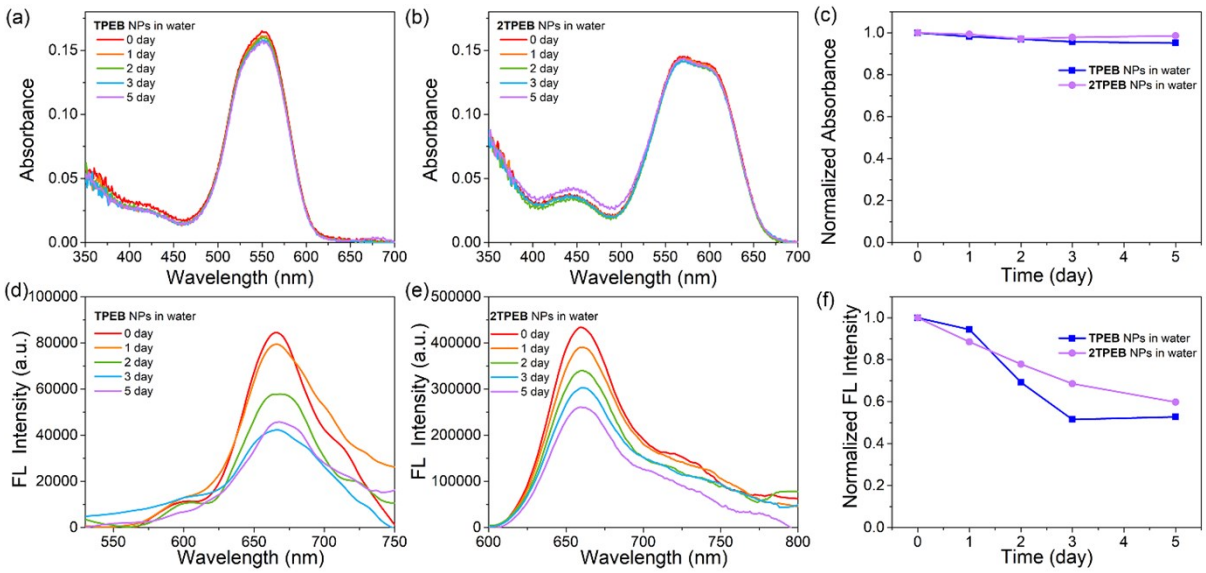


Fig. S21. Absorption spectra of **TPEB** NPs (a) or **2TPEB** NPs (b) dispersed in water recorded at different time intervals; (c) Normalized absorbance spectra of **TPEB** NPs (λ_{max} = 554 nm) or **2TPEB** NPs (λ_{max} = 598 nm) at maximum absorption wavelength dispersed in water recorded at different time intervals. Fluorescence spectra of **TPEB** NPs (d) or **2TPEB** NPs (e) dispersed in water recorded at different time intervals; (f) Normalized fluorescence spectra of **TPEB** NPs (λ_{max} = 660 nm) or **2TPEB** NPs (λ_{max} = 648 nm) at maximum emssion wavelength dispersed in water recorded at different time intervals, excited at 520 nm for **TPEB** and 580 nm for **2TPEB**.

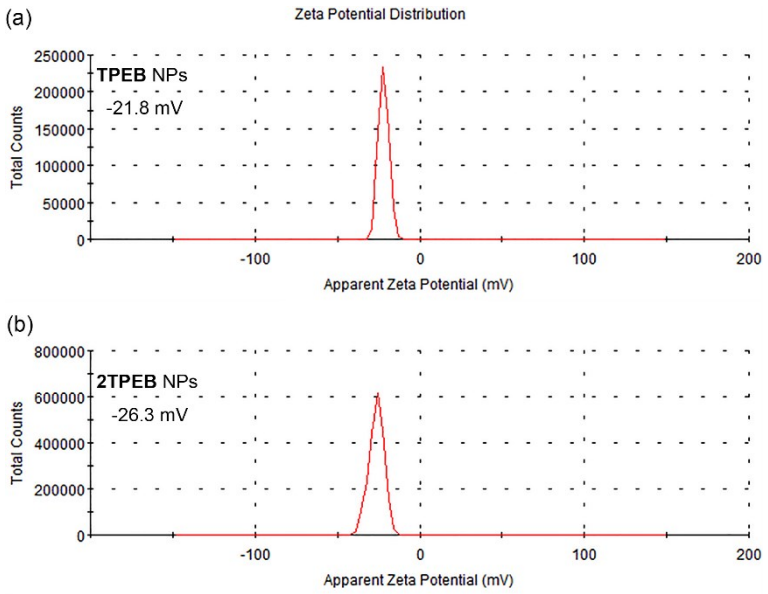


Fig. S22. The Zeta Potential Distribution of **TPEB** NPs or **2TPEB** NPs (1 μM) in aqueous solution.

6. Theoretical calculation

Table S4. Molecular orbital amplitude plots of HOMO and LUMO energy levels of **TPEB** and **2TPEB** at the B3LYP/6-31G(d, p) level by using the Gaussian 09 program package.

□	Electronic transition	TD//B3LYP/6-31G(d, p)			
□		Energy/ eV [a]	*f* [b]	Composition [c]	CI [d]
TPEB	S0→S1	2.2582 eV 549.04 nm	0.5416	HOMO → LUMO	0.6998
	S0→S2	2.8216 eV 439.41 nm	0.4213	HOMO -1 → LUMO	0.6896
	S0→S3	2.9421 eV 421.41 nm	0.0006	HOMO -2 → LUMO	0.7038
2TPEB	S0→S1	2.0754 eV 597.40 nm	0.6159	HOMO → LUMO	0.7019
	S0→S2	2.3639 eV 524.50 nm	0.2610	HOMO -1 → LUMO	0.7053
	S0→S3	2.7343 eV 453.44 nm	0.2543	HOMO -2 → LUMO	0.6967

[a] Only the selected low-lying excited states are presented. [b] Oscillator strength. [c] Only the main configurations are presented. [d] The CI coefficients are in absolute values.

DFT optimized coordinates for TPEB and 2TPEB.

Optimized S0 state Geometry of **TPEB**

B	2.28453700	-0.11605200	1.76346600
C	1.56699700	-0.13235900	-0.78802500
C	2.23473700	-0.08955800	-2.04436900
C	3.58959700	-0.01089400	-1.79868600
C	3.76045100	-0.02840500	-0.39016600
C	4.95102200	-0.07163000	0.34508600
C	4.90806200	-0.23147400	1.73836800
C	5.94304400	-0.37272600	2.69646700
C	5.33165600	-0.56420300	3.93224600
C	3.94240300	-0.53305800	3.70699700
F	1.79196300	1.12096000	2.20120700
F	1.43172000	-1.14347500	2.13779000
N	2.49475100	-0.09340300	0.20804600
N	3.68794600	-0.33552400	2.40013900
H	1.74131100	-0.08303100	-3.00606300
H	4.39267200	0.04542900	-2.52037900
H	7.00188000	-0.34521600	2.47888800
H	5.81255900	-0.71655200	4.88865900
C	0.10992100	-0.20660300	-0.62898700
C	-0.63287700	-0.92357300	-1.58883200
C	-0.59535500	0.43939800	0.40356100
C	-2.01710000	-1.00799100	-1.50757000
H	-0.11548800	-1.44533400	-2.38814200

C	-1.98222000	0.36750000	0.46672100
H	-0.05497700	1.00687000	1.14988400
C	-2.72672600	-0.34970500	-0.48652300
H	-2.55963000	-1.58860700	-2.24773400
H	-2.50217100	0.87595100	1.27233600
C	-4.21098300	-0.47230000	-0.38818200
C	-5.02908900	0.60332100	-0.18583300
C	-4.73193000	-1.86736400	-0.52954500
C	-4.16306700	-2.92042500	0.20887500
C	-5.75829500	-2.17243600	-1.44004000
C	-4.62578100	-4.22820500	0.06509300
H	-3.35756900	-2.70784400	0.90639500
C	-6.21388100	-3.48195500	-1.59281300
H	-6.19617400	-1.37548900	-2.03325700
C	-5.65345300	-4.51469200	-0.83716300
H	-4.18059200	-5.02449200	0.65576100
H	-7.00466600	-3.69598700	-2.30703300
H	-6.00963900	-5.53450000	-0.95510000
C	-6.47272600	0.46113900	0.17479600
C	-7.45571000	1.20153700	-0.50612900
C	-6.88015700	-0.36430000	1.23719400
C	-8.80227100	1.09465300	-0.15949100
H	-7.15942700	1.85876500	-1.31896200
C	-8.22522100	-0.46158800	1.59321200
H	-6.13374200	-0.92701700	1.78926300
C	-9.19305100	0.26257900	0.89282900
H	-9.54637800	1.66524900	-0.70899100
H	-8.51645600	-1.10070100	2.42263100
C	-4.55159700	2.01421200	-0.31254600
C	-4.84468200	2.96032200	0.68598700
C	-3.85083500	2.44581000	-1.45201600
C	-4.42064000	4.28328100	0.56590400
H	-5.40119400	2.65017600	1.56600400
C	-3.43788900	3.77213400	-1.57909200
H	-3.63537300	1.73482300	-2.24378800
C	-3.71533100	4.69511900	-0.56802200
H	-4.64470500	4.99420300	1.35680200
H	-2.90250900	4.08535200	-2.47154100
C	6.27037400	0.01402400	-0.35685700
C	6.91963300	-1.16467500	-0.78053300
C	6.85640700	1.27699200	-0.58633500
C	8.15325200	-1.05431500	-1.43146200
C	8.09149900	1.33519100	-1.23998800
C	8.75900800	0.18327900	-1.66789800
H	8.65203900	-1.96283000	-1.76231800
H	8.54237700	2.30915000	-1.41887000
H	-10.24111600	0.18506400	1.16907700

H	-3.39128600	5.72768200	-0.66560400
H	3.13385300	-0.64084500	4.41767800
C	6.17613600	2.55360900	-0.14421600
H	5.20623200	2.68630600	-0.63792900
H	5.98620100	2.55599600	0.93542500
H	6.79487500	3.42417900	-0.37956500
C	6.30849100	-2.52963600	-0.55287500
H	6.14647000	-2.72643900	0.51319400
H	5.33360000	-2.62263700	-1.04566600
H	6.95962000	-3.31597900	-0.94505400
C	10.10744300	0.27345600	-2.34350000
H	10.19922300	1.19192200	-2.93272900
H	10.91967100	0.27999800	-1.60444900
H	10.28018100	-0.57913000	-3.00828600

SCF done: -2032.01278580 a.u.

No imaginary Frequency.

Optimized S0 state Geometry of **2TPEB**

B	-2.50890500	-0.03146500	0.00445500
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C	-5.36681900	2.46181100	0.13371200
C	-4.83969200	1.15217400	0.01938700
C	-5.52074800	-0.06998000	0.00049500
C	-4.80882200	-1.27438000	-0.01794500
C	-5.30152800	-2.59709900	-0.13512800
C	-4.20319700	-3.42867400	-0.22156300
C	-3.03584700	-2.62074300	-0.14373700
F	-1.71561100	-0.07161700	-1.14407400
F	-1.72521000	0.02776100	1.15880600
N	-3.44473500	1.22910900	0.00515000
N	-3.41247600	-1.31503200	-0.00274500
H	-4.31858200	4.39990400	0.29259300
H	-6.41907400	2.70892800	0.15319800
H	-6.34696200	-2.87157300	-0.15599900
H	-4.20289700	-4.50689600	-0.29720700
C	-1.67032700	-3.15582400	-0.22713600
C	-1.45326000	-4.28835600	-1.03961300
C	-0.57301800	-2.64508900	0.49120900
C	-0.19822400	-4.87515000	-1.13643200
H	-2.27305600	-4.69438000	-1.62407600
C	0.67655600	-3.24934800	0.40445100
H	-0.70215400	-1.77536900	1.12058800
C	0.89404700	-4.38150500	-0.39994700
H	-0.06051400	-5.73283200	-1.78820400
H	1.50361800	-2.83576700	0.97278100
C	2.24287300	-5.00758800	-0.52705800
C	3.01772400	-5.32009000	0.55413500
C	2.67532300	-5.27606400	-1.93384500
C	2.58810900	-4.27098100	-2.91349800
C	3.12084400	-6.54998900	-2.32591900
C	2.96374000	-4.52228500	-4.23299000
H	2.23042600	-3.28373700	-2.63407700
C	3.48552300	-6.80547500	-3.64791400
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C	3.41388600	-5.79139900	-4.60604700
H	2.90110400	-3.72693400	-4.97100600
H	3.82154600	-7.79979800	-3.93022400
H	3.69974100	-5.98986100	-5.63545400
C	4.45614500	-5.70721800	0.42941900
C	4.95220600	-6.83769400	1.10330500
C	5.36021500	-4.92601300	-0.31104800
C	6.29750300	-7.19368000	1.01253300

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C	7.18227000	-6.41251600	0.26457900
H	6.65589100	-8.07951500	1.53032400
H	7.39059500	-4.65103800	-0.96450500
C	2.49091800	-5.31041000	1.95316500
C	3.20190200	-4.66453400	2.98054700
C	1.30770900	-5.99049100	2.28982000
C	2.72787200	-4.66861900	4.29199100
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C	0.83936900	-6.00564000	3.60361900
H	0.75708400	-6.51379500	1.51405000
C	1.54393800	-5.33980600	4.60935800
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H	-0.07470700	-6.54297800	3.84228800
C	-1.75166500	3.11594300	0.22663500
C	-1.56501600	4.25632500	1.03576200
C	-0.64067600	2.63214700	-0.48926000
C	-0.32562600	4.87558600	1.13244900
H	-2.39577800	4.64285700	1.61793700
C	0.59286700	3.26835400	-0.40236800
H	-0.74634600	1.75740800	-1.11597400
C	0.78022100	4.40786000	0.39926500
H	-0.21109300	5.73850700	1.78180600
H	1.43095800	2.87440400	-0.96844900
C	2.11297900	5.06729200	0.52715300
C	2.88280100	5.39358700	-0.55370000
C	2.53568500	5.35115400	1.93386600
C	2.46592800	4.34901900	2.91795300
C	2.95449700	6.63554800	2.32133000
C	2.83273700	4.61378500	4.23729200
H	2.12874900	3.35361400	2.64209700
C	3.31048500	6.90421700	3.64304800
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C	3.25648400	5.89328600	4.60566400
H	2.78389700	3.82071000	4.97878800
H	3.62581700	7.90632500	3.92167700
H	3.53550100	6.10212200	5.63489700
C	4.31182800	5.81358200	-0.42760700
C	4.78407400	6.95177600	-1.10558900
C	5.23138300	5.05612800	0.31850500
C	6.12083300	7.33825000	-1.01327600
H	4.09292100	7.54046600	-1.70230300
C	6.57129900	5.43460800	0.40092700
H	4.89075800	4.16151900	0.83045600
C	7.02101100	6.58053500	-0.25959300

H	6.46058300	8.22952700	-1.53427900
H	7.26580800	4.82976000	0.97801900
C	2.35924000	5.36666500	-1.95366400
C	3.08736700	4.73448000	-2.97755100
C	1.16082600	6.01727000	-2.29482400
C	2.61576400	4.72264900	-4.28983900
H	4.02495700	4.24097600	-2.73725800
C	0.69461600	6.01654300	-3.60944600
H	0.59649800	6.53013300	-1.52195800
C	1.41667300	5.36418000	-4.61160200
H	3.18703200	4.21496700	-5.06254300
H	-0.23161100	6.53101400	-3.85162000
C	-7.01826100	-0.08897500	-0.00094000
C	-7.71915900	-0.16654700	1.22092400
C	-7.71831200	-0.03451600	-1.22513000
C	-9.11794800	-0.18547000	1.19339300
C	-9.11655500	-0.05949500	-1.20114900
C	-9.83659600	-0.13019300	-0.00440100
H	-9.65843900	-0.24697800	2.13568000
H	-9.65623000	-0.02344500	-2.14531000
H	8.23233600	-6.68451100	0.20011700
H	1.17799500	-5.34973200	5.63244500
H	8.06454700	6.87632200	-0.19389400
H	1.05242000	5.36175900	-5.63533700
C	-6.99160000	-0.22919400	2.54573600
H	-6.34955100	-1.11518600	2.61251800
H	-6.34496300	0.64385900	2.69229700
H	-7.70180600	-0.26514100	3.37672800
C	-6.98932500	0.04106300	-2.54847500
H	-6.36671100	0.94076900	-2.61548500
H	-6.32337900	-0.81786600	-2.69215800
H	-7.69838000	0.06010400	-3.38100700
C	-11.34753700	-0.12231500	-0.00640400
H	-11.73707100	0.90441000	-0.00834100
H	-11.74906900	-0.62180300	-0.89444000
H	-11.75181800	-0.62170000	0.88007300

SCF done: -3033.63260258 a.u.

No imaginary Frequency.

7. Imaging studies in cells

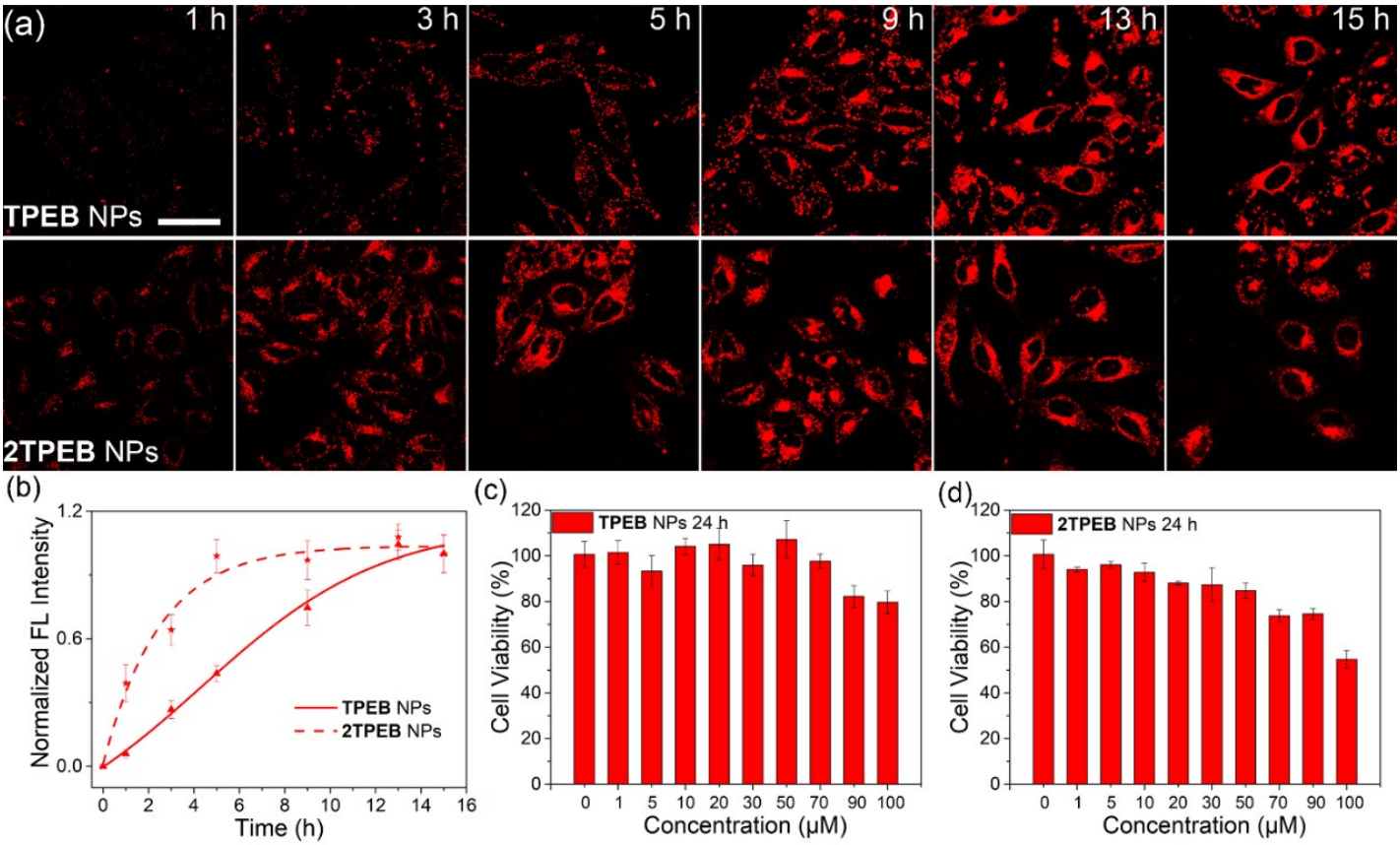


Fig. S23. (a) Fluorescence microscopy images of time-dependent uptake of **TPEB** or **2TPEB** NPs at 10 μM by HeLa cells (human cervical cells); (b) Normalized fluorescence intensity quantitation was analyzed by the images; (c) Cytotoxicity of HeLa cells treated with different concentrations of **TPEB** NPs for 24 h; (d) Cytotoxicity of HeLa cells treated with different concentrations of **2TPEB** NPs for 24 h as demonstrated by CCK-8 assay. Scale bar: 50 μm.

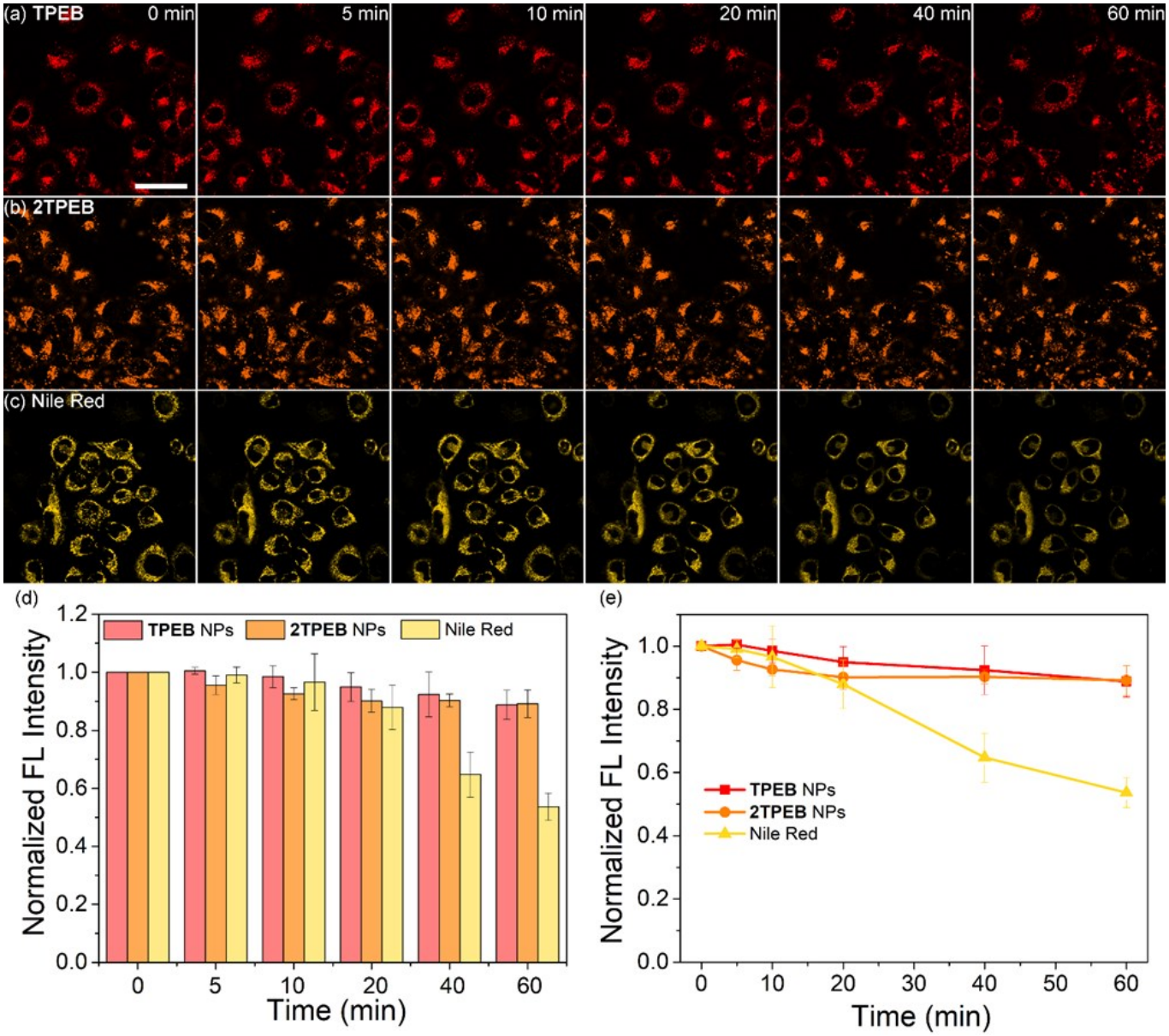


Fig. 24. (a, b, c) Fluorescent images and (d, e) normalized fluorescence intensity of **TPEB** NPs, **2TPEB** NPs or Nile Red by continuous irradiation laser over 60 min with the same laser power of 15%, excited at 552 nm. The image was scanned at different time. Scale bar: 50 μm.

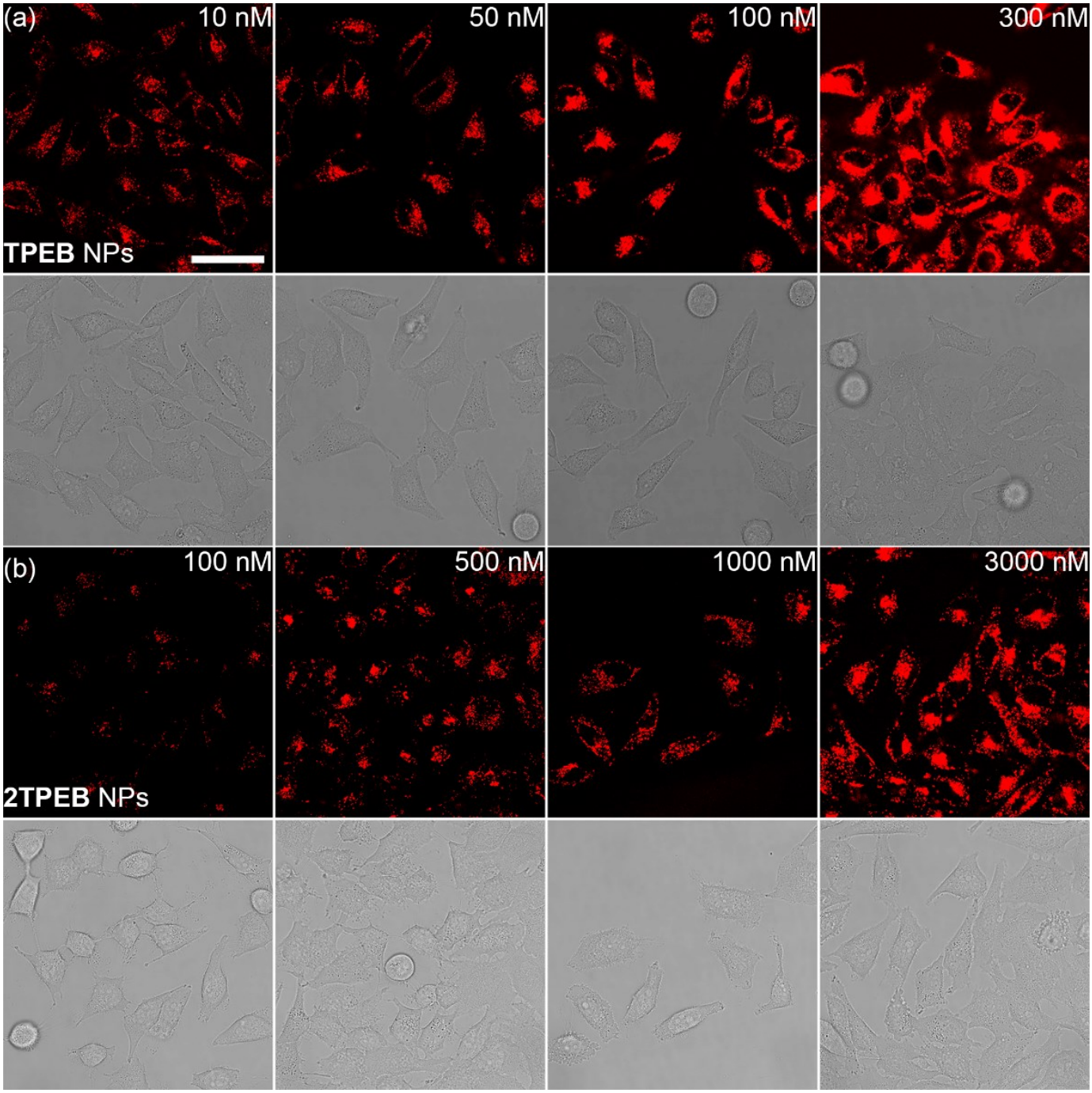


Fig. S25. The concentrations of **TPEB**/**2TPEB** NPs for cell imaging were optimized by HeLa cells. Fluorescence microscope imaging of living HeLa cells with different concentrations of **TPEB** or **2TPEB** NPs for 5 h at 37 ℃. Excited at 552 nm and 638 nm for **TPEB** NPs and **2TPEB** NPs with laser power of 10% or 20%, respectively. Scale bar: 50 μm.

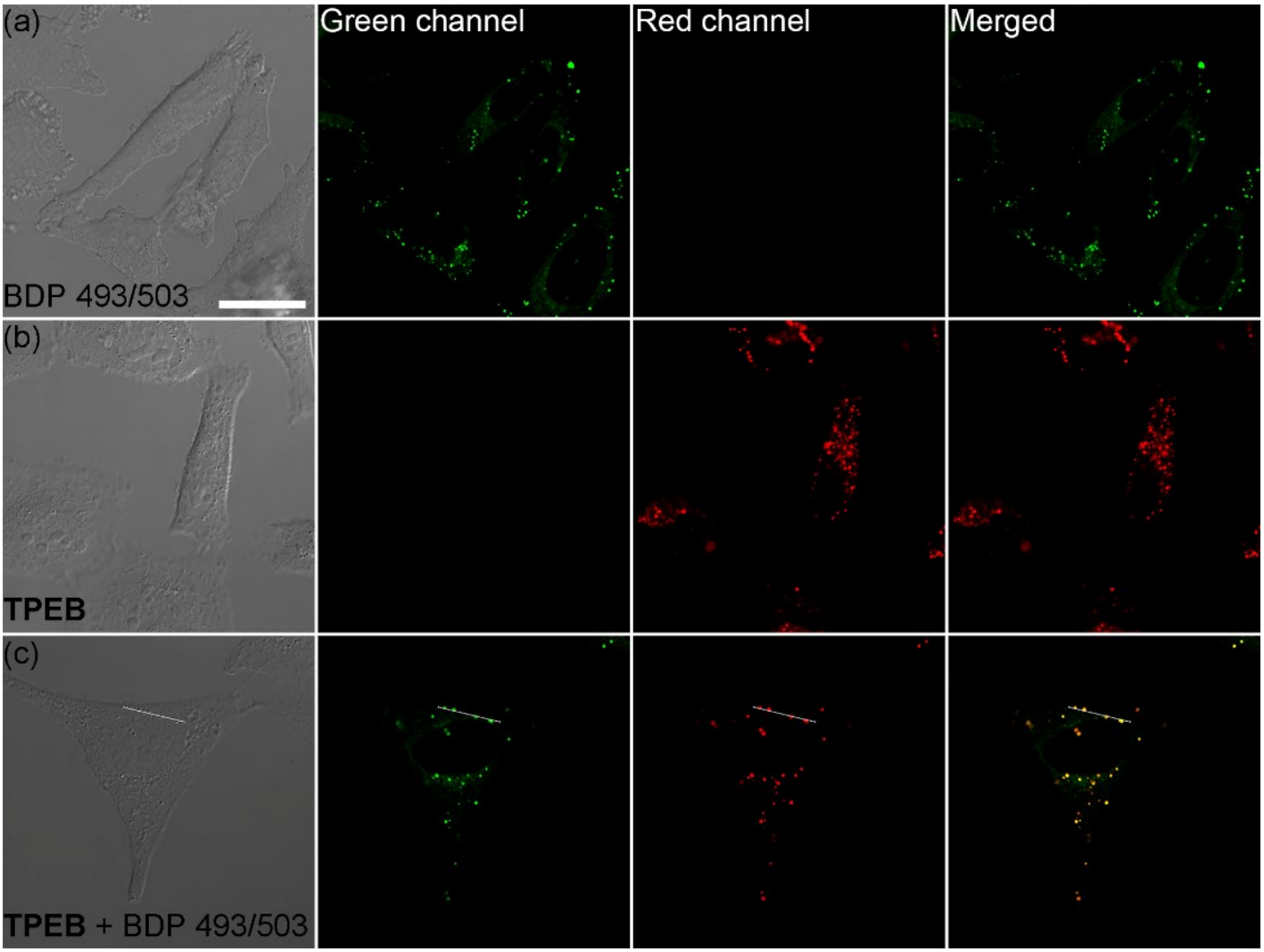


Fig. S26. Co-localization images of HeLa cells stained with BODIPY 493/503 or/and **TPEB** NPs (10 nM). Green channel: fluorescence of BODIPY 493/503 (2 μM, commercial LD-specific probe), excited at 488 nm, and recorded over the spectral regions of 500-550 nm; Red channel: fluorescence of **TPEB** NPs after incubation for 5 h, excited at 552 nm and recorded over the spectral regions of 650-800 nm. Merged images of green and red channel. (a) BODIPY 493/503 stained alone in HeLa cells; (b) **TPEB** NPs stained alone in HeLa cells; (c) BODIPY 493/503 co-stained with **TPEB** NPs in HeLa cells. Scale bars = 25 μm.

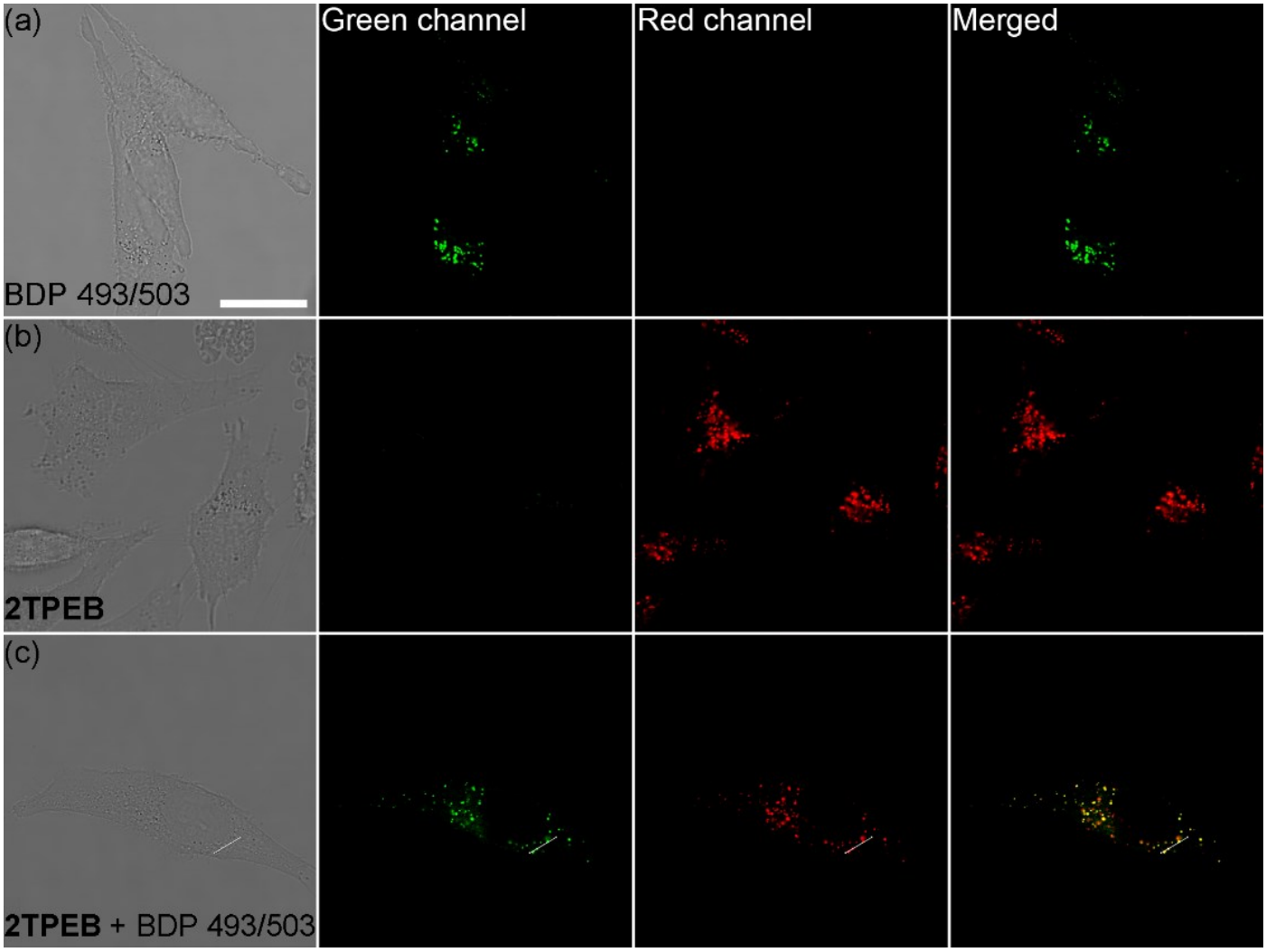


Fig. S27. Co-localization images of HeLa cells stained with BODIPY 493/503 or/and **2TPEB** NPs (100 nM). Green channel: fluorescence of BODIPY 493/503 (2 μM, commercial LD-specific probe), excited at 488 nm and recorded over the 500-550 nm spectral regions; Red channel: fluorescence of **2TPEB** NPs after incubation for 5 h, excited at 638 nm and recorded over the spectral regions of 650-800 nm. Merged images of green and red channel. (a) BODIPY 493/503 stained alone in HeLa cells; (b) **2TPEB** NPs stained alone in HeLa cells; (c) BODIPY 493/503 co-stained with **2TPEB** NPs in HeLa cells. Scale bars = 25 μm.

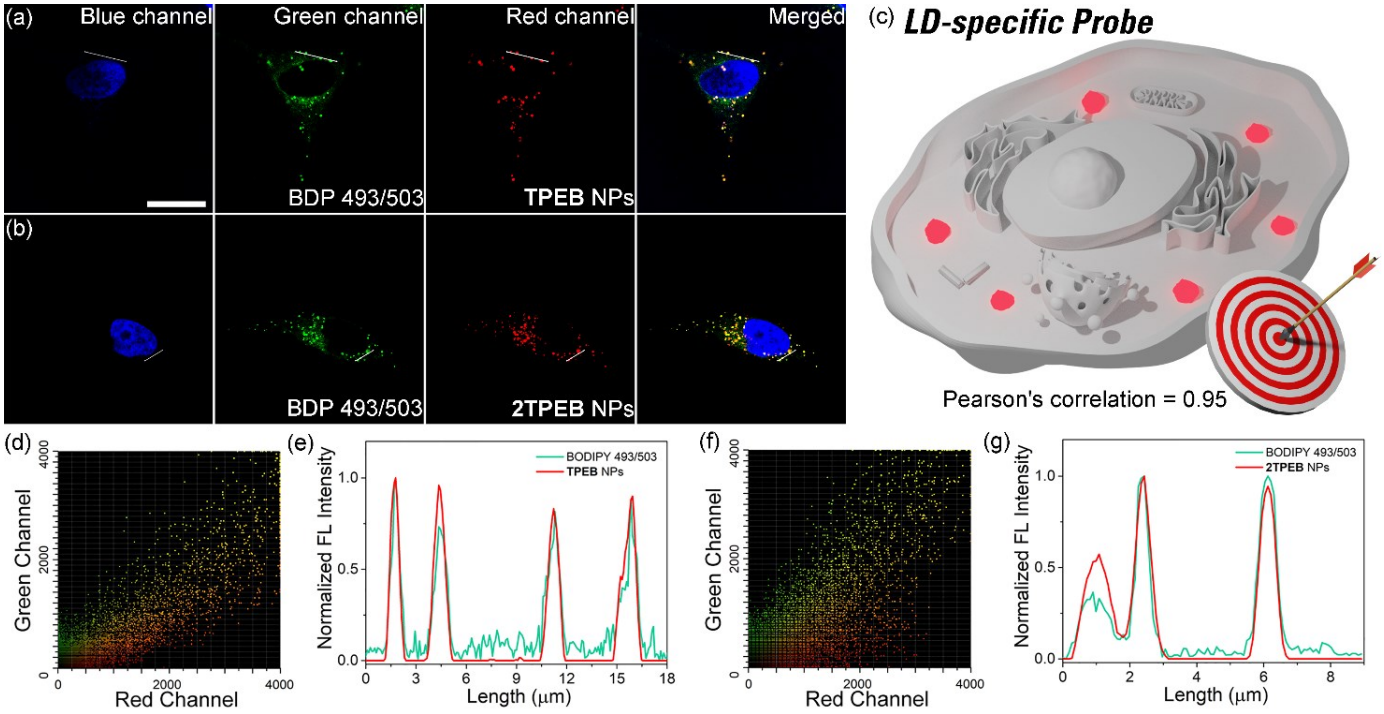


Fig. S28. LD co-localization studies of (a) **TPEB** (10 nM) or (b) **2TPEB** (100 nM) NPs in HeLa cells. Blue channel: 4',6-diamidino-2-phenylindole (DAPI, 0.08 μg/mL) fluorescence showed cell nucleus; Green channel: BODIPY 493/503 (2 μM, lipid droplets commercial dye) fluorescence, λ_{ex} = 488 nm; Red channel: **TPEB**/**2TPEB** NPs fluorescence, λ_{ex} = 552 nm for **TPEB** and 638 nm for **2TPEB**. Merged images of blue, green and red channel. (c) The schematic of LD localization of **TPEB**/**2TPEB** NPs. (d, f) Correlation scatter diagram of BODIPY 493/503 and **TPEB**/**2TPEB** NPs intensities, Scale bars = 25 μm; (e, g) Intensity profiles within the regions of interests of **TPEB**/**2TPEB** NPs and BODIPY 493/503 across Hela cells, Pearson's correlation Rr = 0.95/0.95.

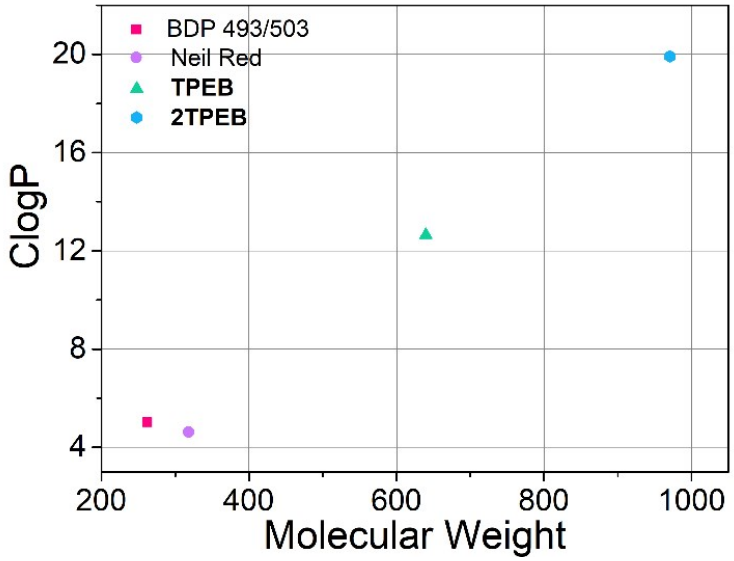


Fig. S29. The calculated logP values of BODIPY 493/503, Nile Red, **TPEB**, and **2TPEB**, estimated by using ChemBioDraw Ultra, version 14.0.

The preparation of TPEB/2TPEB aggregates and BODIPY 493/503 NPs

TPEB/**2TPEB** aggregates: **TPEB**/**2TPEB** aggregates were prepared by a nanoprecipitation method. A certain amount of **TPEB**/**2TPEB** (5 μL, 2 mM) in high-purity solvent DMSO were injected into 1640 complete medium (1 mL). The sudden solubility decreases in culture medium resulted in self-assembly of **TPEB**/**2TPEB** into corresponding aggregates (10 μM, 1 mL).

BODIPY 493/503 NPs: Chloroform solution of BODIPY 493/503 (44 μL, 566 μM) and chloroform solution of F127(30 μL, 7.1 mg mL^{-1}) were added to a flask contained 1 mL chloroform. The obtained mixture was then dried under vacuum in a rotary evaporator to remove the chloroform completely. After that, deionized water or culture medium (5 mL) was added, and the flask was placed under sonication for several minutes to give the aqueous dispersion of BODIPY 493/503 NPs (5 μM, 5 mL).

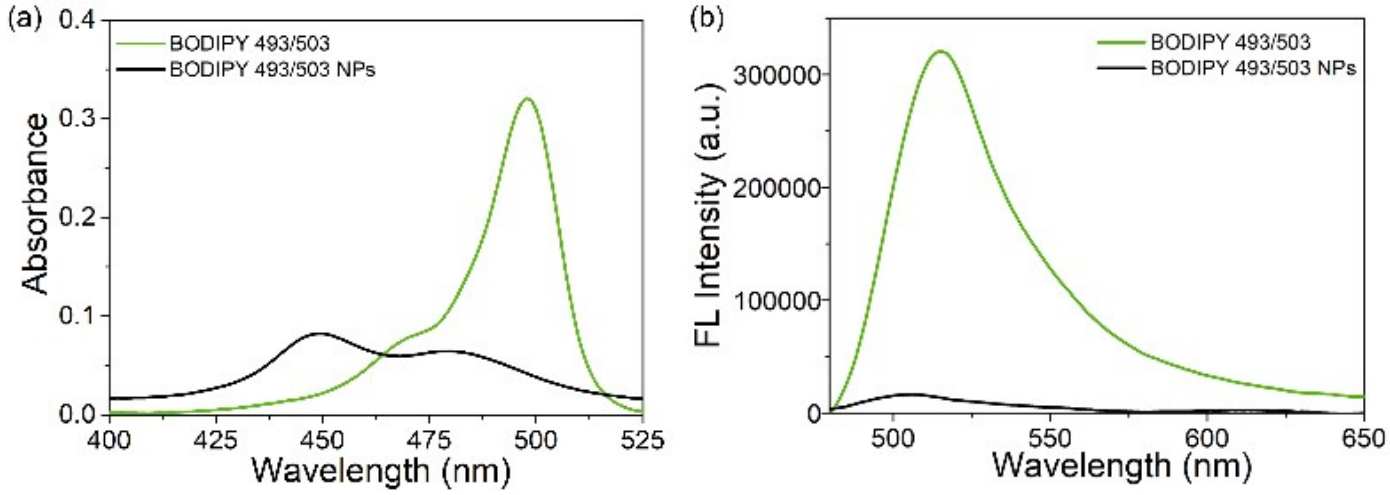


Fig. S30. (a) Absorption and (b) fluorescence spectra of BODIPY 493/503 (in chloroform, green line) or BODIPY 493/503 NPs wrapped with F127 (in deionized water, black line) at 5 μM, excited at 480 nm.

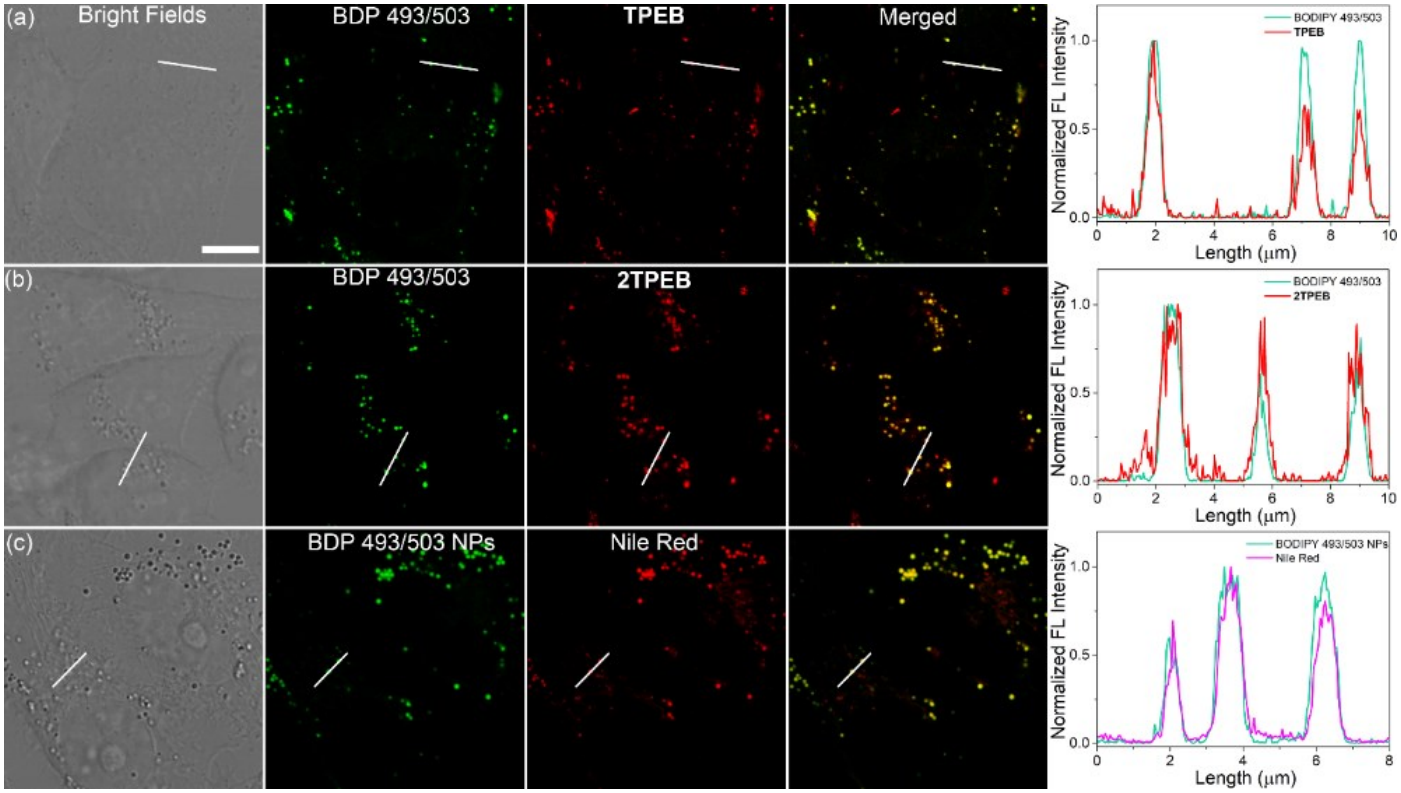


Fig. S31. LD co-localization studies of **TPEB**, **2TPEB** or BODIPY 493/503 NPs in HeLa cells. (a-b) **TPEB** or **2TPEB** aggregates (10 μM) were prepared by the above steps. The obtained **TPEB**/**2TPEB** aggregates were co-incubated with BODIPY 493/503 (2 μM) in HeLa cells for 3 h. Intensity profiles within the regions of interests of **TPEB**/**2TPEB** NPs and BODIPY 493/503 across HeLa cells, Pearson's correlation Rr = 0.94/0.92, respectively. (c) BODIPY 493/503 wrapped with F127 NPs (5 μM) were prepared by the above steps. The obtained BODIPY 493/503 & F127 NPs were co-incubated with Nile Red (1 μM) in Hela cells for 3 h. Pearson's correlation Rr = 0.97.

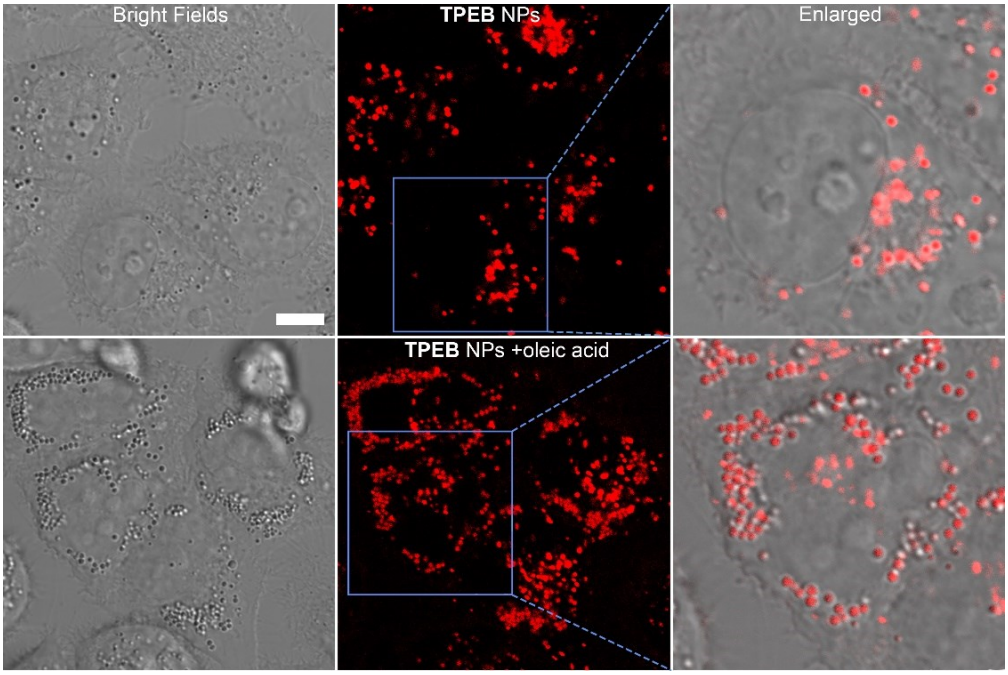


Fig. S32. CLSM images of HeLa cells pretreated with oleic acid and labeled with **TPEB** NPs. The upper row images: cells treated with **TPEB** NPs (0.1μM). The bottom row images: cells pretreated with oleic acid (200 μM) for 4 h, subsequently incubated with **TPEB** NPs (0.1μM). The pictures were captured at the same voltage strength. Scale bar: 10 μm.

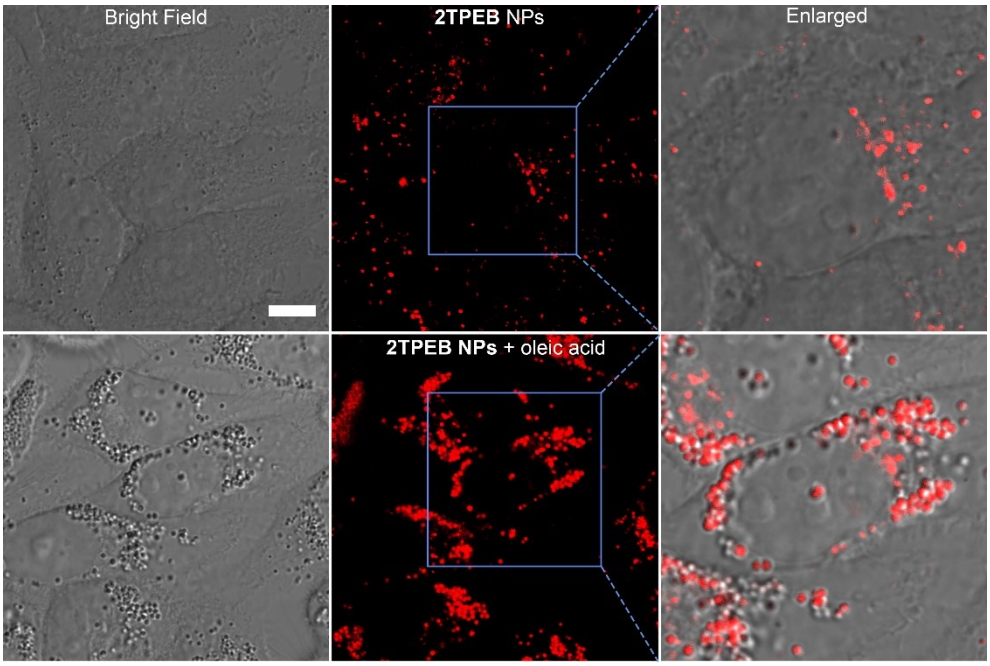


Fig. S33. CLSM images of HeLa cells pretreated with oleic acid and labeled with **2TPEB** NPs. The upper row images: cells treated with **2TPEB** NPs (1μM). The bottom row images: cells pretreated with oleic acid (200 μM) for 4 h, subsequently incubated with **2TPEB** NPs (1μM). The pictures were captured at the same voltage strength. Scale bar: 10 μm

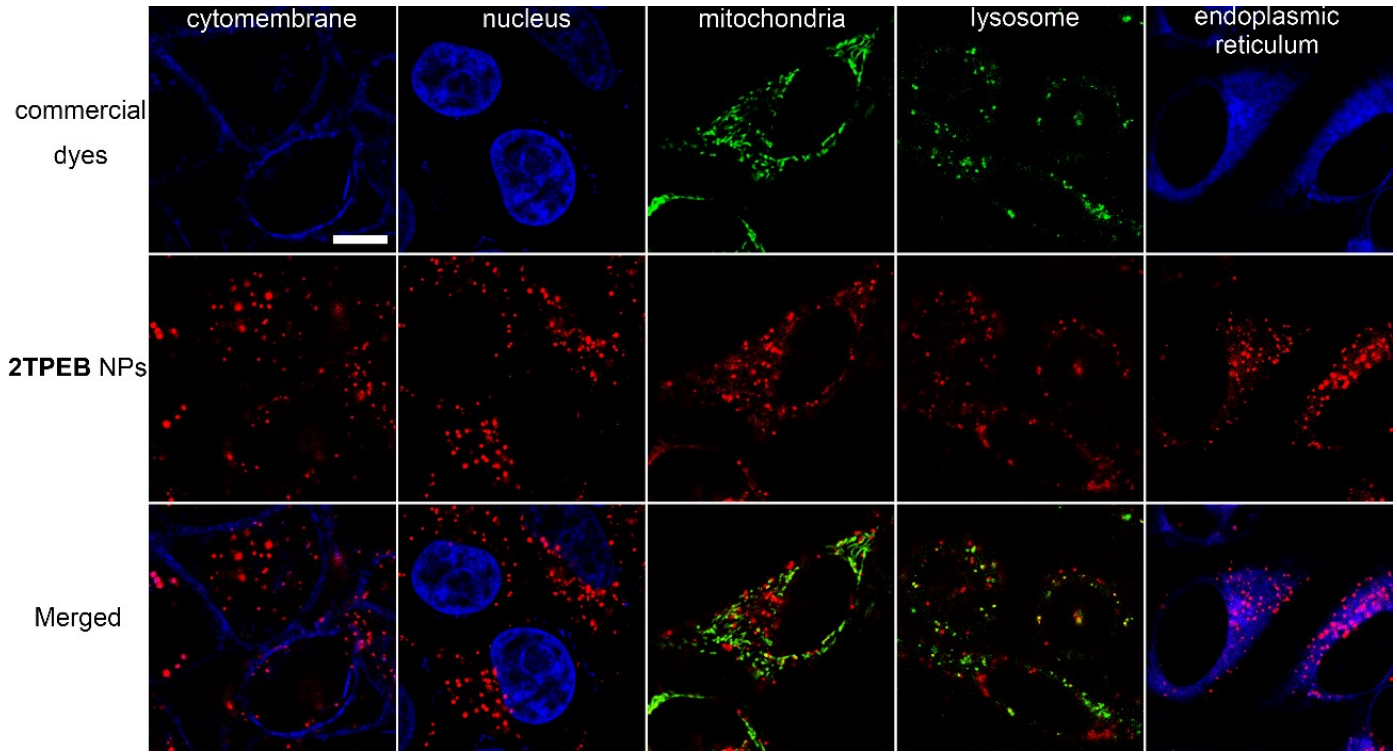


Fig. S34. Multicolor images of **2TPEB** NPs with commercial organelle dyes in Hela cells. The upper row images represent organelle dyes (cytomembrane: Laudan, 1 μM; nucleus: DAPI, 0.0161 μg mL^{-1}; mitochondria: Rhodamine 123, 10 μM; lysosome: LysoTracker™ Green DND-26, 1 μM; endoplasmic reticulum: ER-Tracker Blue-White DPX, 0.5 μM) and the middle row are **2TPEB** NPs. The bottom row images represent merged images. Scale bar is 10 μm.

The calculation of Two-photon cross section and cellular imaging

The two-photon-induced fluorescent spectra of **TPEB** or **2TPEB** were determined with the same excitation wavelength (from 700 to 1000 nm, every 10 nm) using fluorescein as the reference by femtosecond two-photon excited fluorescence (TPEF) technique. The Two-photon cross section was calculated by using the below equation according to previous reports.[8]

$$\delta_2 = \frac{\delta_1 \Phi_1 (F_2 c_1)}{\Phi_2 (F_1 c_2)}$$

In which the subscripts 1 and 2 refer to the reference of fluorescein and our probe **TPEB** or **2TPEB**, respectively; F is the integration of the fluorescent spectra; Φ is the fluorescence quantum yield (Φ = 0.95 for fluorescein, NaOH solution, pH =11; Φ = 0.66 for **TPEB**, Φ = 0.78 for **2TPEB** in n-hexane solution); c is the concentration of fluorescein (100 μM) and our sample (100 μM); and δ_1 is the TPA cross-section of the reference of fluorescein according to previous reports.[9]

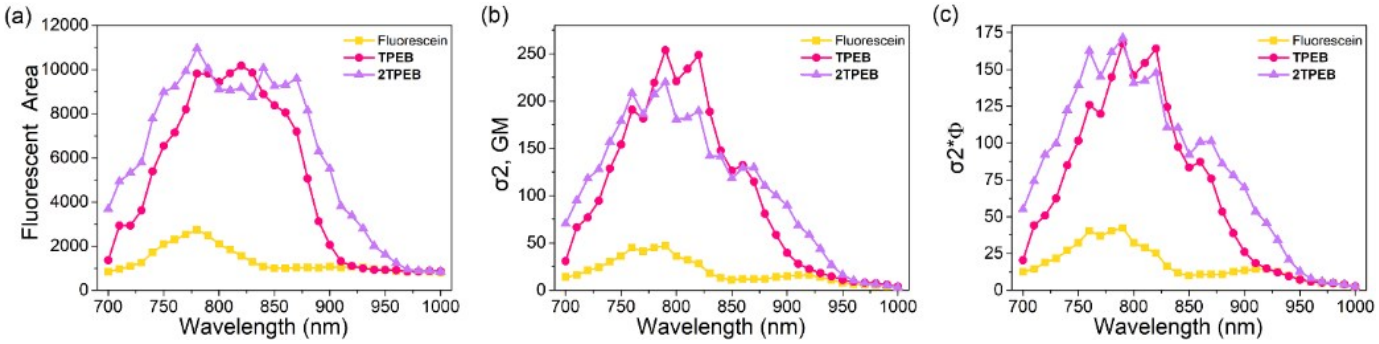


Fig. S35. (a) The fluorescent integration area of **TPEB**, **2TPEB** (in n-hexane solution) or fluorescence (NaOH solution, pH =11) at different excitation wavelength. (b) The calculated Two-photon cross section spectra by the above equation. (c) The activity Two-photon cross section spectra fluorescence spectra of **TPEB**, **2TPEB** or fluorescence at different excitation wavelength.

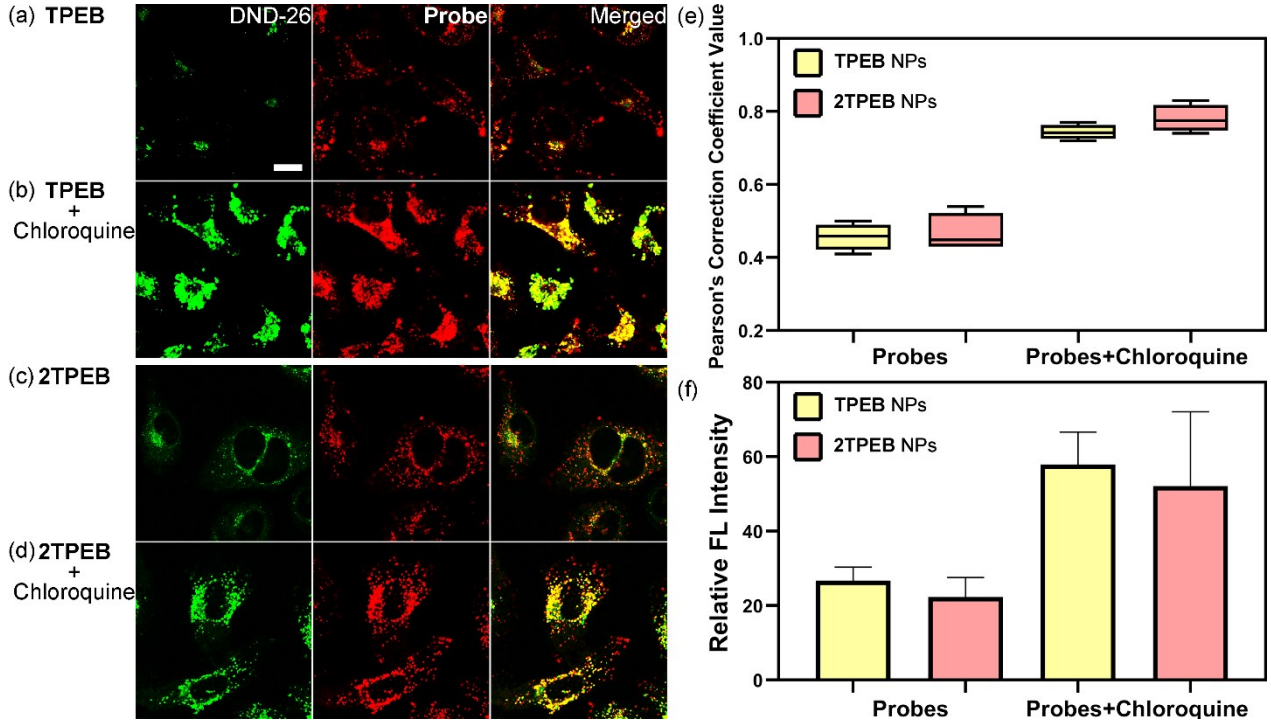
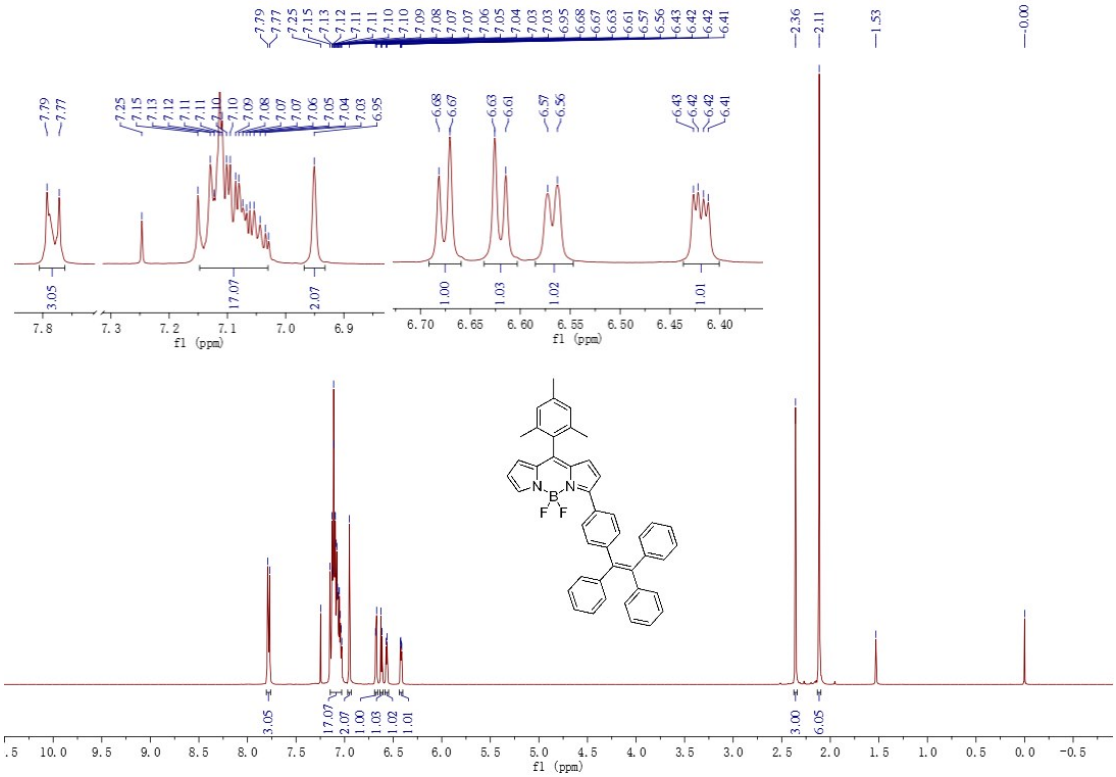
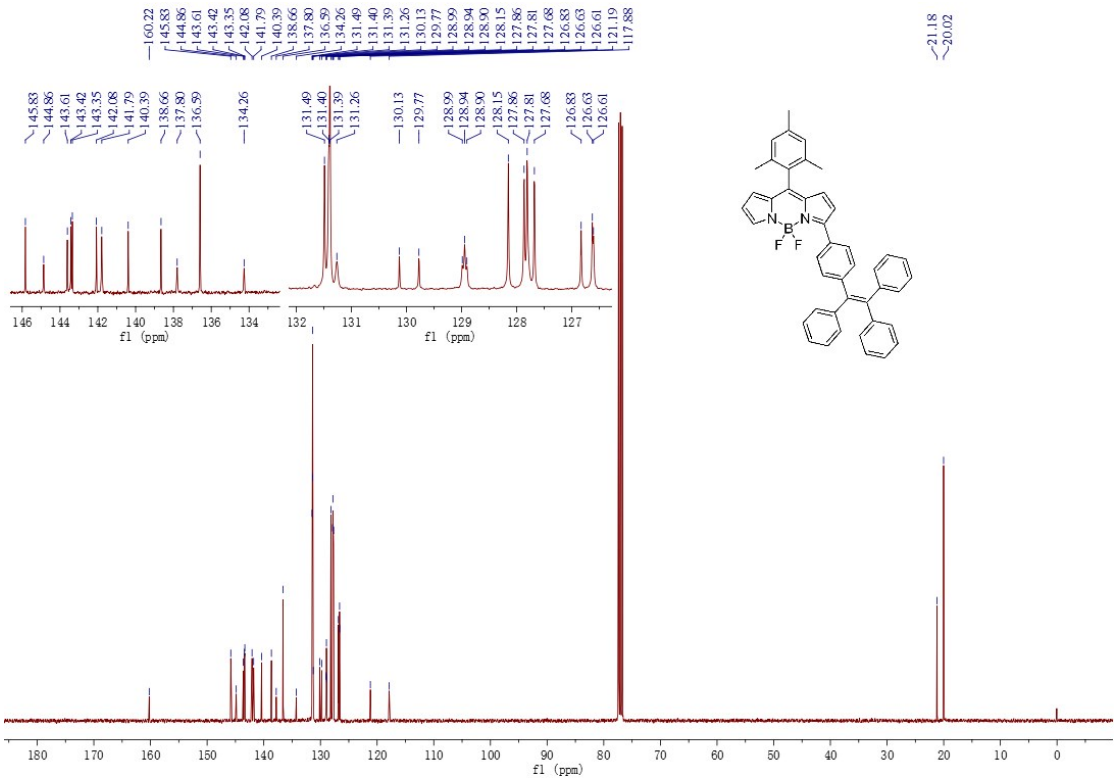


Fig. 36. CLSM images of HeLa cells untreated or treated with indicated inducers of phospholipidosis (chloroquine) and labeled with **TPEB**/**2TPEB** NPs and LysoTracker® Green DND-26. (a), (c) Cells treated with **TPEB** or **2TPEB** NPs and DND-26 (1 μM). (b), (d) Cells pretreated with chloroquine (200 μM) for 8 h, subsequently incubated with **TPEB** (0.1 μM) or **2TPEB** (1 μM) NPs and DND-26 (1 μM). (e) Pearson's correlation coefficient for **TPEB**/**2TPEB** NPs and DND-26. (f) Fluorescence intensities of **TPEB**/**2TPEB** acquired from fluorescent images. λ_{ex}: 488 nm and λ_{em}: 500-540 nm for DND-26 (green); λ_{ex}: 522 nm or 638 nm and λ_{em}: 650−800 nm for **TPEB**/**2TPEB** NPs (red). Scale bar: 10 μm.

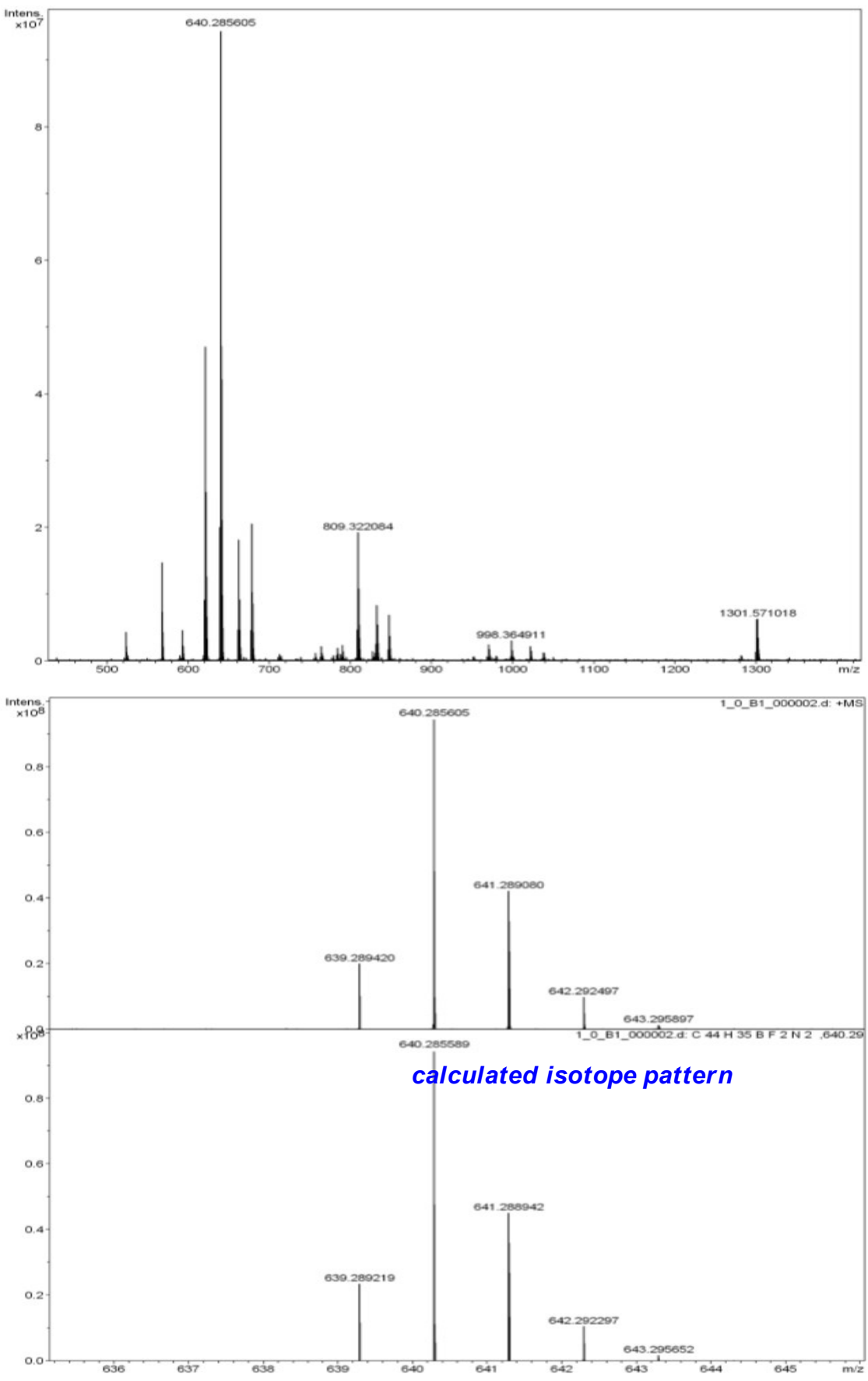
8. ^{1}H, ^{13}C and HRMS spectra for all compounds



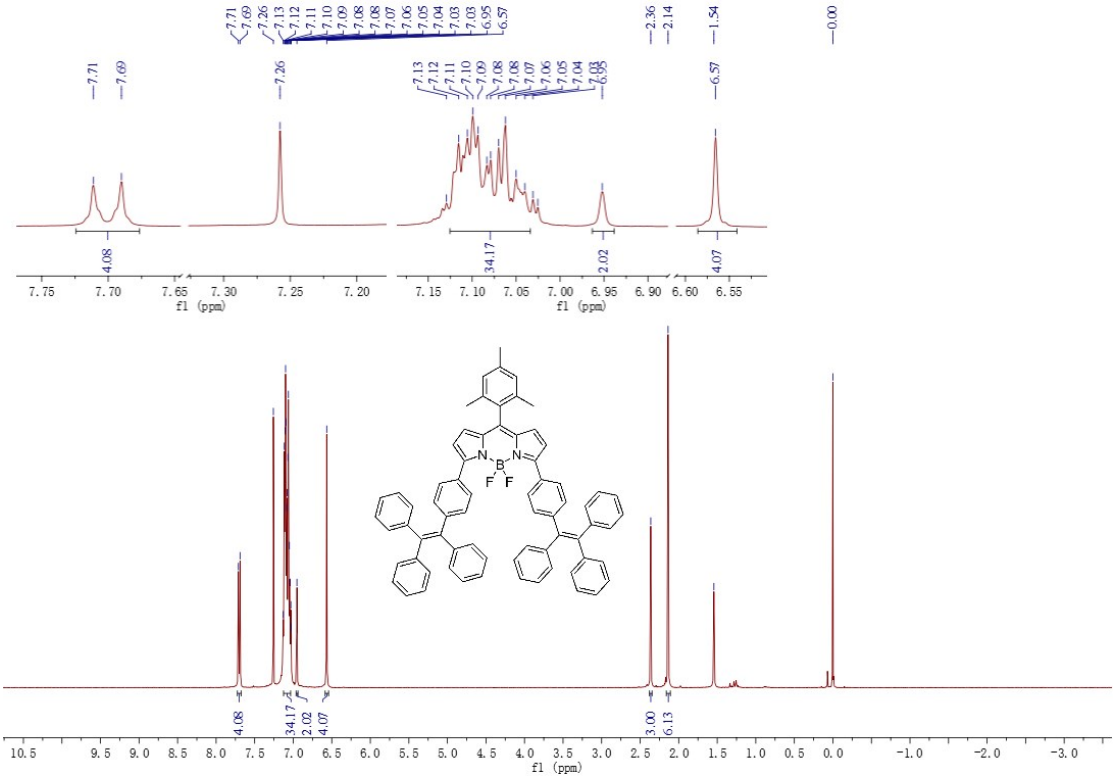
^{1}H NMR spectrum of **TPEB** in $CDCl_3$



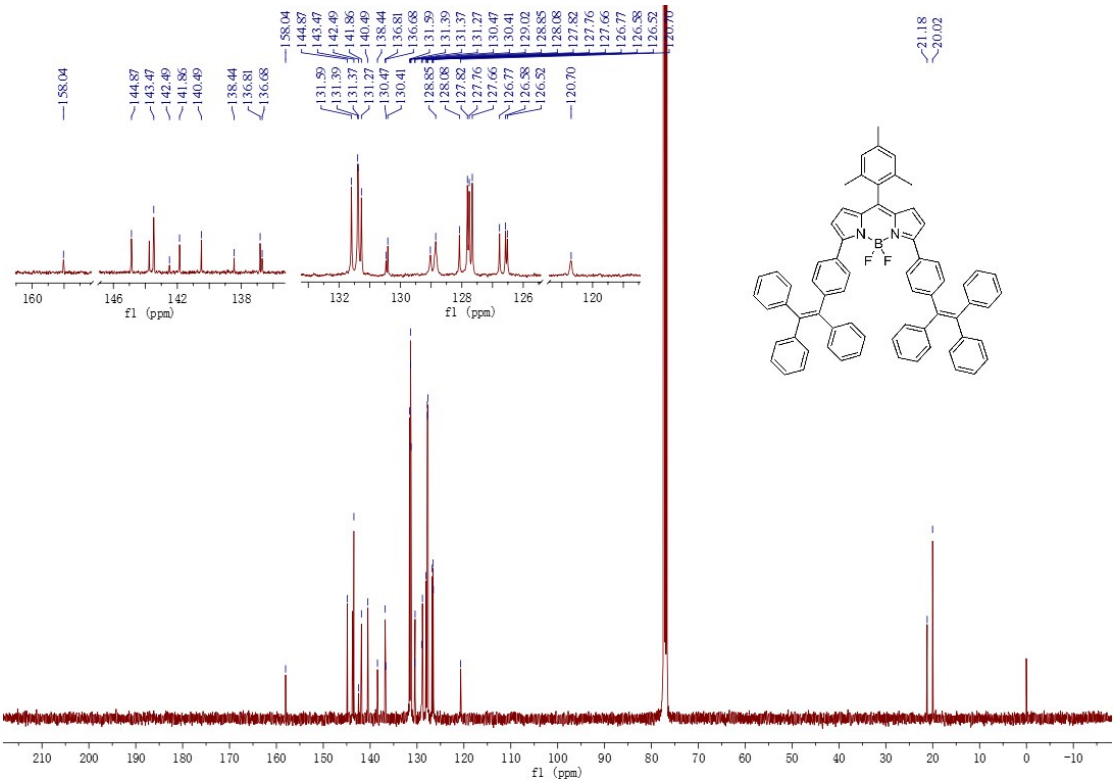
^{13}C NMR spectrum of **TPEB** in $CDCl_3$



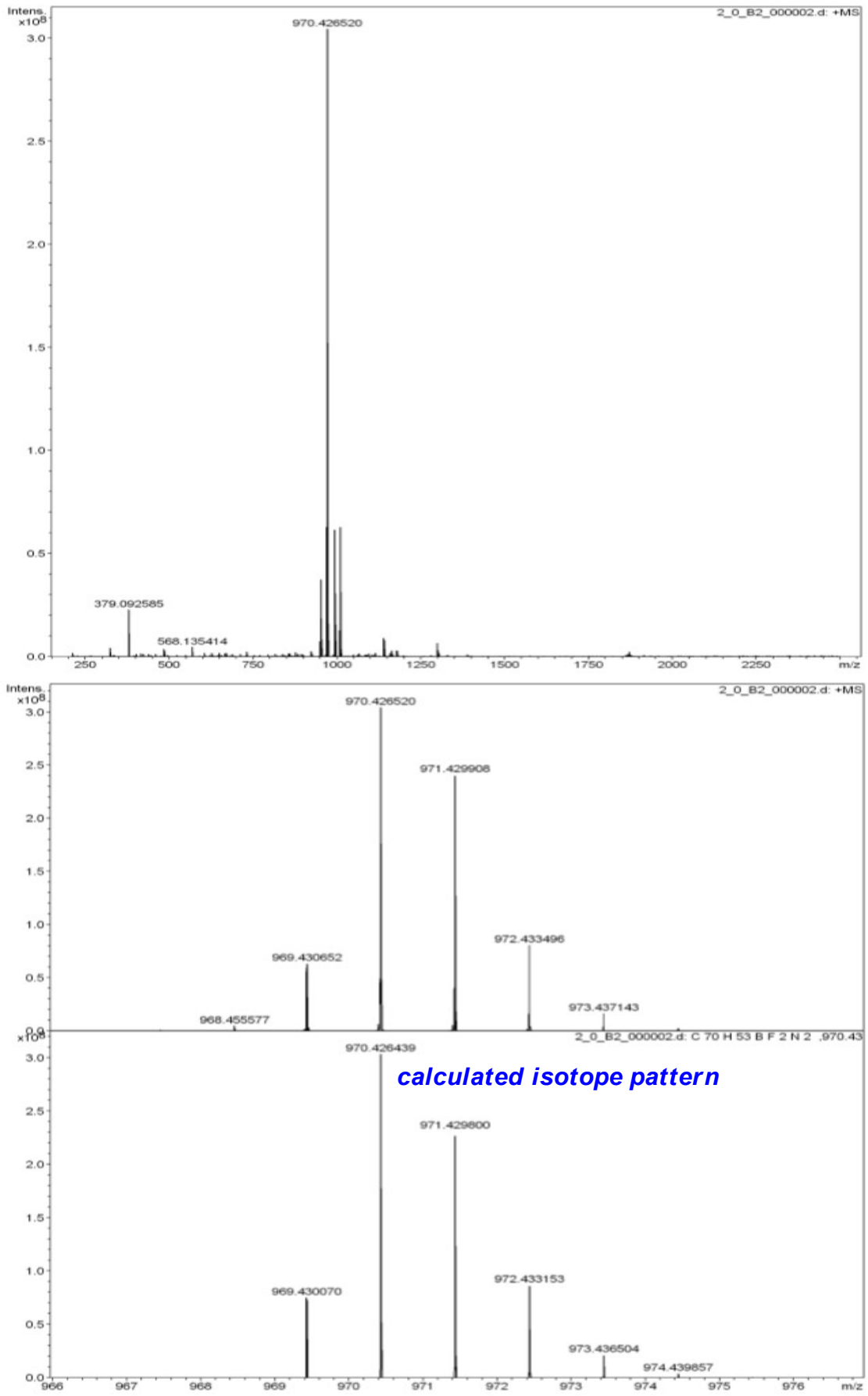
HRMS for **TPEB**



^{1}H NMR spectrum of **2TPEB** in $CDCl_3$



^{13}C NMR spectrum of **2TPEB** in $CDCl_3$



HRMS for **2TPEB**

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