Cell membrane-targeting AIE photosensitizer as necroptosis inducer for boosting cancer theranostics

Niu Niu,^{†ab} Ying Yu,^{†c} Zhijun Zhang,^{ab} Miaomiao Kang,^{ab} Lei Wang,^{ab} Zhen Zhao,^{*c} Dong Wang^{*ab} and Ben Zhong Tang^{*c}

Table of Contents

Sch	eme	S1. Synt	hesis	routine of	TBMPE and T	BMPEI			S 1
Fig. S1		S1	$^{1}\mathrm{H}$	NMR	spectrum	of	TBMPE	in	CDCl ₃ .
Fig.	S2 ¹	³ C NMR	l spec	trum of TE	MPE in CDCl	3.			S2
Fig.	S3 H	IRMS sp	pectru	m of TBM	PE.				S2
Fig.	S4 ¹	H NMR	spect	rum of TB	MPEI in CDC	3.			S3
Fig.	S5 ¹	³ C NMR	l spec	trum of TE	MPEI in CDC	l ₃ .			S3
Fig.	S6 H	IRMS sp	pectru	m of TBM	PEI.				S4
Fig.	S7 A	AIE curv	e of T	TBMPE in '	THF/H ₂ O mixt	ure.			S4
Fig.	S8 A	Absorptio	on and	d emission	spectra of TBN	MPE and	d TBMPEI in	solid stat	tes. S4
Fig.	S9 F	Fluoresce	ence s	pectra chai	nge of DCFH v	vith diff	erent photose	nsitizers.	S5
Fig.	S10	Absorpt	tion sp	pectra chan	ge of ABDA v	vith diff	erent photose	nsitizers.	S5
Fig.	S11	Fluores	cence	spectra cha	ange of HPF w	ith diffe	erent photoser	nsitizers.	S5
Fig.	S12	Fluores	cence	spectra cha	ange of DHR 1	23 with	different pho	otosensitiz	zers. S5
Fig.	S13	DFT cal	lculati	ion of TBN	IPE and TBM	PEI.			S6
Fig.	S14	CLSM i	mage	of cells sta	nined for differ	ent time	2.		S6
Fig.	S15	CLSM i	mage	of cells sta	ined with TB	MPE.			S 6
Fig.	S16	Photosta	ability	of TBMP	EI.				S 7
Fig.	S17	Cytotox	icity	of TBMPE	towards 4T1 c	ells.			S7
Fig.	S18	MDA re	elease	assay of 4	T1 cells after c	lifferent	treatment.		S 7
Fig.	S19	CLSM i	mage	s of cell sta	ained with Hoe	chst 33	342 with irrad	liation.	S 7
Fig.	S20	CLSM i	mage	s of cells a	fter long time	light irra	adiation.		S8
Fig.	S21	Live/De	ad sta	aining of 47	Γ1 cells.				S 8
Fig.	S22	LDH re	lease	of 4T1 cell	s after various	treatme	nt.		S9
Fig.	S23	Charact	erizat	ion of TBN	IPEI dots.				S9
Fig.	S24	Ex vivo	fluore	escence image	age of mice aft	er 24 h	injection.		S10
Fig.	S25	Photos of	of tun	nors after 1	4-days treatme	nt.			S10
Fig.	S26	Body-w	eight	change of	mice.				S10
Fig.	S27	H&E sta	aining	g of mice m	ajor organs in	differen	t groups.		S10
Fig.	S28	Hemoly	tic as	say of TBN	IPEI dots.				S10
Fig.	S29	Quantur	n yiel	d and lifeti	me of TBMPE	and TE	BMPEI		S10

Supporting Figures



Scheme S1. Synthesis routine of TBMPE and TBMPEI.



Fig. S1 ¹H NMR spectrum of TBMPE in CDCl₃.



Fig. S2 ¹³C NMR spectrum of TBMPE in CDCl₃.



Fig. S3 HRMS spectrum of TBMPE.



Fig. S4 ¹H NMR spectrum of TBMPEI in CDCl₃.



210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

Fig. S5 ¹³C NMR spectrum of TBMPEI in CDCl₃.



Fig. S6 HRMS spectrum of TBMPEI.



Fig. S7 (A) Fluorescence spectrum of TBMPE (10 μ M) in THF/H₂O mixture with different water fraction. (F) Plots of relative PL intensity (I/I_0) of TBMPE versus water fraction.



Fig. S8 Absorption and emission spectrum of TBMPE and TBMPEI in solid states.



Fig. S9 Fluorescence spectrum of (a) DCFH (10 μ M) and in the presence of (b) TBMPE (1 μ M), (c) TBMPEI (1 μ M), (d) Rose bengal (1 μ M), (e) Ce 6 (1 μ M) in PBS after irradiation for different time. Ex: 490 nm.



Fig. S10 Absorption spectrum of (a) ABDA (20 μ M) and in the presence of (b) TBMPE (2 μ M), (c) TBMPEI (2 μ M), (d) Rose Bengal (2 μ M), (e) Ce 6 (2 μ M) in PBS after irradiation for different time.



Fig. S11 Fluorescence spectrum of (a) HPF (10 μ M) and in the presence of (b) TBMPE (2 μ M), (c) TBMPEI (2 μ M), (d) Rose bengal (2 μ M), (e) Ce 6 (2 μ M) in PBS after irradiation for different time. Ex:500 nm.



Fig. S12 Fluorescence spectrum of (a) DHR 123 (10 μ M) and in the presence of (b) TBMPE (2 μ M), (c) TBMPEI (2 μ M), (d) Rose Bengal (2 μ M), (e) Ce 6 (2 μ M) in PBS after irradiation for different time. Exi: 490 nm.

	TBMEP					TBMEPI				
	eV		eV	ΔE_{ST}		eV		eV	ΔE_{ST}	
S5	4.0325	Т5	3.1608	0.8717	S5	2.8946	Т5	2.8464	0.0482	
S4	3.7934	Т4	2.9876	0.8058	S4	2.8435	Т4	2.3960	0.4475	
S3	3.5085	Т3	2.5886	0.9199	S3	2.6593	Т3	2.2664	0.3929	
S2	3.2379	Т2	2.4405	0.7974	S2	2.2088	Т2	1.9398	0.269	
S1	2.3425	T1	1.1389	1.2036	S1	0.9496	T1	0.9101	0.0395	

Fig. S13 DFT calculations of molecular energy at various states and responding ΔE_{st} . Calculation was performed by DFT theory calculations at the m062x/6-31g* level using the Gaussian 09 program.



Fig. S14 CLSM images of 4T1 cells after incubation with TBMPEI for various time periods. Scale bar: $10 \ \mu m$.



Fig. S15 CLSM images of 4T1 cells after incubation with TBMPE (10 μ M, 30 min) and then costained with lysosome blue (2 μ M, 30 min). Scale bar: 10 μ m.



Fig. S16 Photostability comparison of TBMPEI (5 μ M) and Cell Mask Green (2 μ M). (a) CLSM images of 4T1 cells stained with TBMPEI and Cell Mask Green before and after 50 loops of 488 nm laser (1% intensity) irradiation.



Fig. S17 Cytotoxicity of TBMPE towards 4T1 cells.



Fig. S18 MDA contents of 4T1 cells after different treatment.



Fig. S19 CLSM images of 4T1 cells stained with Hoechst 33342 (2 μ M) and under continuous 488 nm laser (3% intensity) irradiation for different loops. Scale bar: 10 μ m.



Fig. S20 CLSM images of 4T1 stained with TBMPEI (10 μ M) and Hoechst 33342 (2 μ M) and then irradiated with white LED lamp for various time periods. Scale bar: 10 μ m.



Fig. S21 Live/dead staining of 4T1 cells by FDA (6 μ M, green fluorescence) and PI (10 μ M, red fluorescence) after various treatments. Scale bar: 50 μ m.



Fig. S22 LDH release of 4T1 cells after treated with various amounts of TBMPEI, 4T1 cells were chosen as the control.



Fig. S23 Particle size distributions of TBMPEI dots in aqueous solution. Inset: TEM image of TBMPEI dots. Scale bar: 100 nm.



Fig. S24 *Ex vivo* fluorescence images of tumors and major organs after injection of TBMPEI dots for 24 h.









Fig. S26 Body weights change of mice in different groups during 14-day treatments.

Fig. S27 H&E-stained images of tissue sections (kidney, lung, spleen, liver, and heart) of mice treated with PBS, PBS + light, TBMPEI dots, and TBMPEI dots + light. Scale bar: 200 μ m.



Fig. S28 Hemolysis assay of TBMPEI dots. (A) Photographs of Hemolysis assay using various materials and (B) corresponding hemolysis rates.

AIEgens	ф	F	α _{AIE} (I _{aggr,max} /I _{solu})	τ [ns]
	Soln (ϕ_{F})	Aggr (ϕ_F)		
TBMPE	1.8%	3.6%	2	1.11
TBMPEI	0.4%	0.6%	1.5	2.31

Fig. S29 Fluorescence quantum yield and lifetime of TBMPE and TBMPEI.