Electronic Supplementary Information (ESI)

Photoactivatable tandem fluorescence imaging of organelles and their interplay monitoring

Zicong Zhang,[†] Wenping Pan,[†] Yayu Xie,[‡] Kai Liu,[‡] Meng Gao,[†*] and Yingjun Wang[†*]

[†]National Engineering Research Center for Tissue Restoration and Reconstruction, Key Laboratory of Biomedical Engineering of Guangdong Province, Key Laboratory of Biomedical Materials and Engineering of the Ministry of Education, Innovation Center for Tissue Restoration and Reconstruction, School of Biomedical Science and Engineering, School of Materials Science and Engineering, South China University of Technology, Guangzhou 510006, P. R. China.

[‡]Institute of Marine Drugs, Guangxi University of Chinese Medicine, Nanning 530200, China.

[*]Corresponding author: msmgao@scut.edu.cn; imwangyj@scut.edu.cn

Table of contents

Materials and chemicals

5-(4-(diphenylamino)phenyl)thiophene-2-carbaldehyde, piperidine, sodium borohydride ($NaBH_4$) and solvents were purchased from Henan Psai Chemical Products Co., Ltd., Aladdin, Sigma, Guangzhou Chemical Reagent Factory and used as received without further purification. Dulbecco's Modified Essential Medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (Life Technologies). Ultra-pure water was supplied by Milli-Q Plus System (Millipore Corporation, United States). Phosphate buffered saline (PBS), penicillin and streptomycin were purchased from Thermo Fisher Scientific. Cholesterol, lecithin, 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA), 9,10-anthracenediyl-bis(methylene) dimalonic acid (ABDA), Chlorin e6 (Ce6), Annexin V-FITC and propidium iodide (PI) were purchased from Sigma-Aldrich. Mito-Tracker Deep Red (MTDR) was purchased from Invitrogen. Nile Red, tetramethylrhodamine ethyl ester perchlorate (TMRE) were purchased from MCE. Methylene blue trihydrate was purchased from Sinopharm Chemical Reagent Co., Ltd.. 4% Paraformaldehyde fix solution was purchased from Beyotime Biotechnology.

Equipment and methods

UV−vis absorption spectra were measured on a Shimadzu UV-2600 spectrophotometer, medium scanning rate, and quartz cuvettes of 1 cm path length. Photoluminescence (PL) spectra were recorded on Shimadzu RF-6000 spectrofluorometer. The absolute fluorescence quantum yield was measured using a Hamamatsu absolute fluorescence quantum yield spectrometer C11347 Quantaurus QY. The ^{1}H and ^{13}C NMR spectra were measured on a Bruker AV 400 NMR spectrometer. High resolution mass spectra (HRMS) were recorded on an Aglient 7250 & JEOL-JMS-T100LP AccuTOF mass spectrometer operated in ESI model. Confocal laser scanning microscopic (CLSM) images were obtained on the confocal microscope (Zeiss; LSM880; Germany). Cell viability assay was conducted on a microplate reader (Tecan Infinite M200 PRO)

Table S1. The photophysical properties of compound THTTVP

		λ_{ex} [nm][d)]	λ_{em} [nm][e)]	Φ_f [%][f)]	τ [ns][g)]	k_r [10^7 s^{-1}][h)]	k_{nr} [10^8 s^{-1}][i)]
THTTVP	PBS[a)]	371	502	1.95	2.83	0.69	3.46
	1,4-dioxane[b)]		486	35.5	2.20	16.1	2.93
TTVP[1]	PBS[a)]	480	–	–	–	–	–
	Liposome[c)]		581	27.0	3.13	8.6	7.93

a) THTTVP or TTVP in PBS with a concentration of 10 μM; b) THTTVP in water/1,4-dioxnae mixtures with 90% 1,4-dioxane volume fraction at a concentration of 10 μM; c) TTVP in liposome solution (1.0 mg/mL) with a concentration of 10 μM; d) Maximum absorption wavelength; e) Maximum emission wavelength; f) Absolute quantum yield; g) Average fluorescence lifetime; h) Radiative relaxation rate $k_r = \Phi/\tau$; i) Non-radiative relaxation rate $k_{nr} = (1-\Phi)/\tau$.

Table S2. Molecular coordinates of optimized THTTVP

#Molecular charge and spin multiplicity were 1 and 1, respectively.

C	-1.08274915	-0.48132155	-0.88690605
C	-2.42807194	0.06672454	-0.93399260
C	-3.52529284	-0.62674364	-1.26112502
C	-4.88063947	-0.09353517	-1.29871865
C	-5.90846179	-0.89804853	-1.58307682
C	-7.33600182	-0.45966967	-1.58480523
N	-7.52195445	0.83892873	-0.95985557
C	-6.52437378	1.78406475	-1.43501900
C	-5.12966101	1.36073045	-1.00525529
C	-8.87112756	1.34537223	-1.13743118
C	-9.93055454	0.52703230	-0.40119623
C	-9.60968347	0.48662833	1.08048907
C	-0.63204317	-1.75336272	-1.12626823
C	0.76492406	-1.88921640	-0.97582164
C	1.39449640	-0.72622474	-0.62009474
S	0.24143281	0.54436361	-0.47887443
C	2.82394893	-0.50388329	-0.38565480
C	3.28400137	0.49565278	0.47247748
C	4.63429514	0.68626822	0.69583770
C	5.58001650	-0.11437106	0.05690931
C	5.12896784	-1.11147880	-0.80825679
C	3.77775344	-1.30167742	-1.02080241
N	6.95539463	0.07995593	0.27641350
C	7.47451439	1.38569339	0.42751525
C	7.82835088	-1.02946787	0.34330237
C	8.42639462	1.65510583	1.40868521
C	8.94338941	2.93278977	1.54797493
C	8.50807641	3.96240319	0.72540021
C	7.55436068	3.69742252	-0.24761717
C	7.04627675	2.41832179	-0.40436226
C	9.06690721	-0.98972580	-0.29362673
C	9.92618243	-2.07371139	-0.21795473
C	9.55817511	-3.21717651	0.47723323
C	8.32163483	-3.26128746	1.10614775
C	7.46475155	-2.17434966	1.04947250
N	-10.49804613	-0.39979926	1.91866535
C	-11.93538806	-0.05328636	1.72948708
C	-10.28296505	-1.83643733	1.58157365
C	-10.13447940	-0.18706767	3.35076573
H	-2.51999514	1.11685530	-0.67747370
H	-3.43226147	-1.68000072	-1.51080096

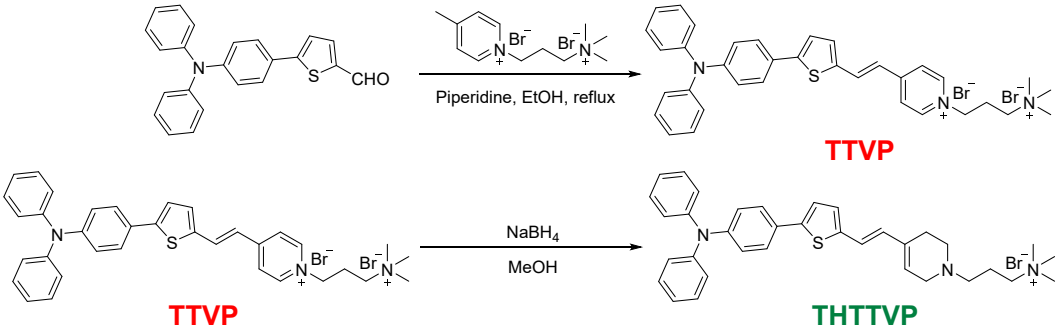
H	-5.73018965	-1.94124854	-1.82183498
H	-7.71301860	-0.45808849	-2.62600763
H	-7.92700190	-1.21013035	-1.05324007
H	-6.75369619	2.76792685	-1.02402094
H	-6.55987142	1.87477330	-2.53481053
H	-4.99157442	1.55057797	0.06355965
H	-4.39722442	1.97931264	-1.52968631
H	-9.14940828	1.39057911	-2.20297155
H	-8.89382053	2.36967297	-0.75865738
H	-9.98783364	-0.47550076	-0.82566404
H	-10.89416017	1.00490909	-0.57737555
H	-9.69139431	1.48603753	1.50701522
H	-8.59127313	0.13529230	1.23671738
H	-1.28272286	-2.57389702	-1.39428174
H	1.28961705	-2.82562755	-1.10505136
H	2.57547472	1.12169120	1.00185351
H	4.95982115	1.46002671	1.37847255
H	5.84520283	-1.73595410	-1.32580904
H	3.46022612	-2.06734910	-1.71740681
H	8.76187151	0.85822411	2.05988214
H	9.68325467	3.12585488	2.31464241
H	8.90855500	4.96112670	0.84096323
H	7.21069397	4.48934889	-0.90121468
H	6.31162331	2.21540364	-1.17293039
H	9.35480573	-0.10456207	-0.84606798
H	10.88557903	-2.02704833	-0.71777648
H	10.22846578	-4.06523806	0.52893584
H	8.02421515	-4.14408635	1.65808766
H	6.50669143	-2.21019192	1.55190839
H	-12.23595259	-0.30326512	0.71699879
H	-12.52402351	-0.62875843	2.43874273
H	-12.06596149	1.01071959	1.91012330
H	-10.56287585	-2.01352121	0.54823488
H	-10.90557876	-2.44037693	2.23592159
H	-9.23445706	-2.07792298	1.73447612
H	-9.07709181	-0.40264169	3.47879937
H	-10.34078762	0.84639495	3.61525934
H	-10.73015592	-0.85573605	3.96620045

Table S3. Molecular coordinates of optimized TTVP

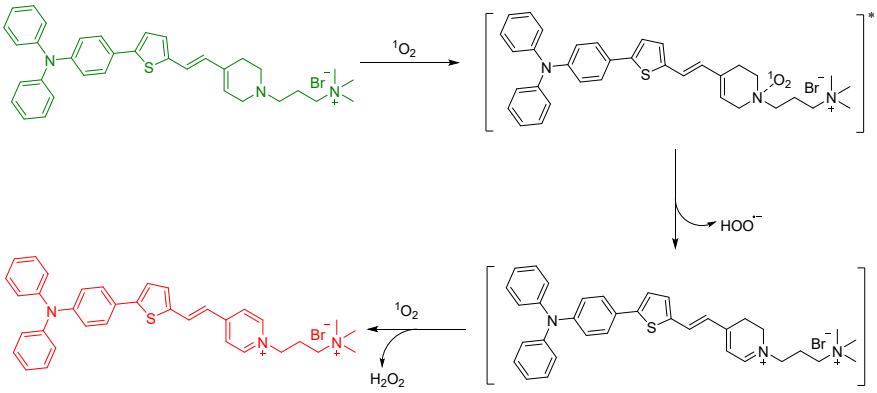
#Molecular charge and spin multiplicity were 2 and 1, respectively.

C	0.94051553	1.14808511	-0.15448240
C	2.29412745	0.80186515	-0.42602303
C	3.36546095	1.60565168	-0.22105075
C	4.72398133	1.24881270	-0.49958243
C	5.13322369	0.00184331	-1.01275799
C	6.45145062	-0.24881188	-1.25477560
H	6.79614234	-1.19392330	-1.65164488
N	7.40072195	0.67937266	-1.01521352
C	7.05219121	1.88220255	-0.52024890
H	7.85691528	2.58422028	-0.34933676
C	5.74676846	2.18592748	-0.26007798
H	5.51131734	3.16695777	0.13091286
C	8.81041765	0.35774190	-1.24249429
C	9.42969066	-0.28554351	-0.00821466
C	10.89025887	-0.57035366	-0.28874667
C	0.40040919	2.32388898	0.33909818
C	-0.98738424	2.28251329	0.46654672
C	-1.54606945	1.07641121	0.07298904
S	-0.31610250	-0.00006817	-0.44568270
C	-2.94712864	0.69839138	0.07278738
C	-3.41853686	-0.39530934	-0.66267805
C	-4.75147065	-0.74571649	-0.66007298
C	-5.68051668	-0.01520502	0.08948566
C	-5.21740076	1.07801780	0.83098740
C	-3.88361098	1.42476563	0.81892415
N	-7.02925661	-0.36691413	0.09793229
C	-8.02876898	0.62070895	0.24092838
C	-7.42106289	-1.71699157	-0.03768454
C	-9.10962727	0.40343495	1.09345876
C	-10.09990783	1.36437293	1.22106674
C	-10.01949447	2.55678443	0.51364421
C	-8.94017747	2.77557135	-0.33280401
C	-7.95403973	1.81300635	-0.47719000
C	-6.76447801	-2.71989184	0.67373433
C	-7.15742699	-4.04152102	0.53713691
C	-8.21756138	-4.37899651	-0.29445925
C	-8.87869294	-3.37887152	-0.99548632
C	-8.48154812	-2.05665170	-0.87575727
N	11.63415624	-1.26862191	0.81708975
C	13.05391774	-1.40764247	0.38098411
C	11.59537877	-0.47533083	2.07715926

C	11.07459458	-2.62666620	1.06579110
H	2.45167402	-0.19358033	-0.83067262
H	3.21477652	2.60220313	0.18054797
H	4.42522075	-0.78479639	-1.23135156
H	9.32209506	1.28336494	-1.50610779
H	8.86285049	-0.30868972	-2.10344918
H	8.88219282	-1.20187542	0.21980837
H	9.31602873	0.39532950	0.83718867
H	11.43110765	0.35861096	-0.47490170
H	10.99731957	-1.20587610	-1.16918867
H	0.99793529	3.19066527	0.58922285
H	-1.57912061	3.11815030	0.81460835
H	-2.73359251	-0.96982457	-1.27749463
H	-5.08518022	-1.58730422	-1.25392096
H	-5.91321116	1.65031095	1.43166024
H	-3.55930377	2.25947829	1.42878071
H	-9.16987823	-0.52320681	1.65207178
H	-10.93489285	1.18284138	1.88827067
H	-10.79258846	3.30865952	0.61943859
H	-8.87037015	3.69793818	-0.89821165
H	-7.11990526	1.98032651	-1.14842302
H	-5.94589680	-2.45759503	1.33362094
H	-6.63898850	-4.81139897	1.09735527
H	-8.52692639	-5.41272034	-0.39389714
H	-9.70479943	-3.62919135	-1.65138852
H	-8.99358087	-1.27839004	-1.42935730
H	13.47034393	-0.41581694	0.21939070
H	13.60919962	-1.92418864	1.16039368
H	13.08367120	-1.98181280	-0.54245170
H	11.95924528	0.52947924	1.87299236
H	12.23609732	-0.96290297	2.80841747
H	10.57690497	-0.44009330	2.45347281
H	10.05766874	-2.53959637	1.43776578
H	11.09039894	-3.18986229	0.13510140
H	11.69252391	-3.12061738	1.8122290



Scheme S1. Synthetic routes to TTVP and THTTVP



Scheme S2. The speculated photoactivation mechanism of THTTVP

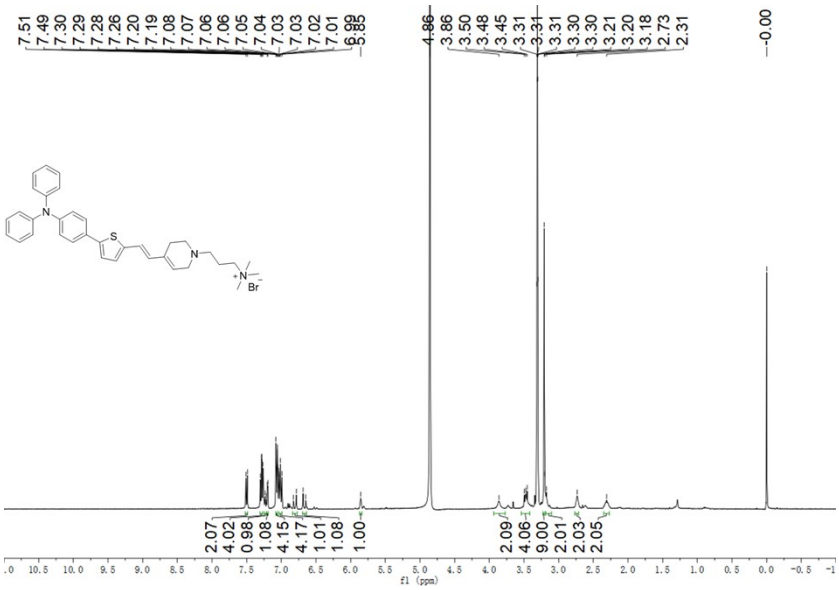


Figure S1. ^{1}H NMR spectrum of THTTVP in CD_3OD.

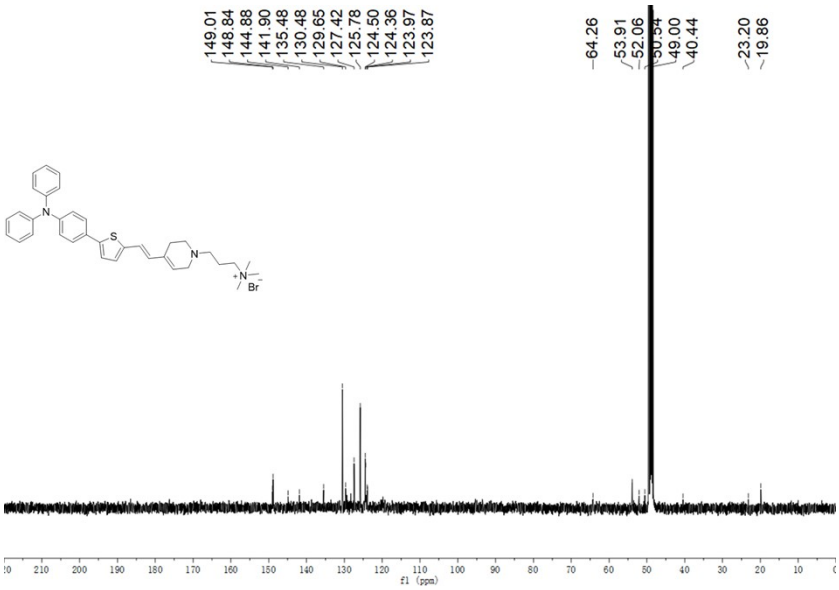


Figure S2. ^{13}C NMR spectrum of THTTVP in CD_3OD.

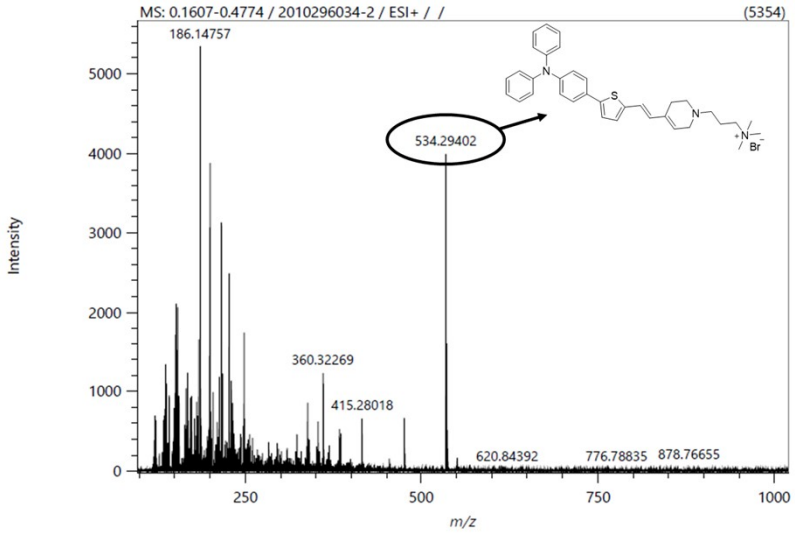


Figure S3. HRMS spectrum of THTTVP.

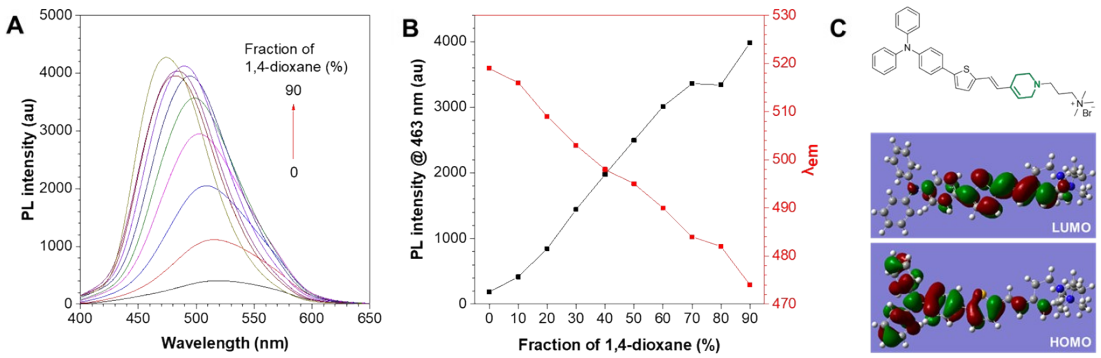


Figure S4. (A) The PL spectra and (B) plots of PL intensity at 463 nm and maximum emission wavelength (λ_{em}) of THTTVP in 1,4-dioxane/water mixtures with increasing fraction of 1,4-dioxane. (C) Molecular orbital amplitude plots of HOMO and LUMO energy levels of THTTVP. λ_{ex} = 371 nm.

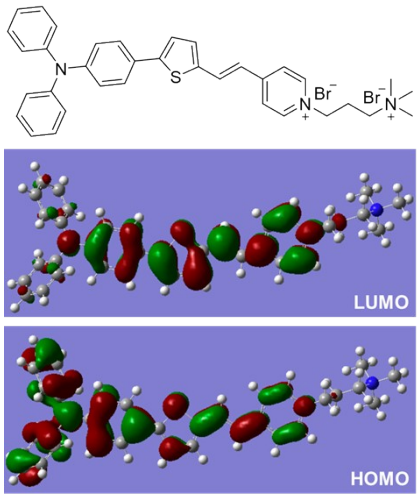


Figure S5. Molecular orbital amplitude plots of HOMO and LUMO energy levels of TTVP.

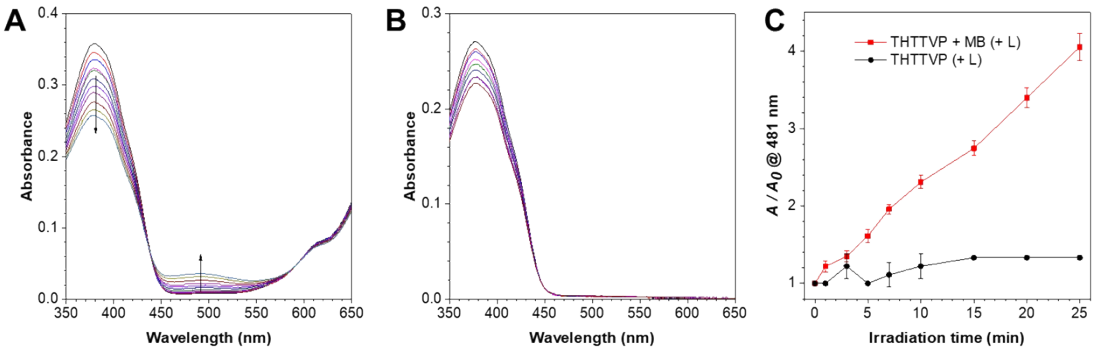


Figure S6. The UV−vis absorption spectra of THTTVP (A) with or (B) without methyl blue (MB) under continuous 660 nm light irradiation (30 mW/cm^2) for 0−25 min.[1] (C) Plots of relative absorption wavelength at 481 nm (A/A_0@481 nm). [THTTVP] = 20 μM; [Methyl blue] = 2 μM.

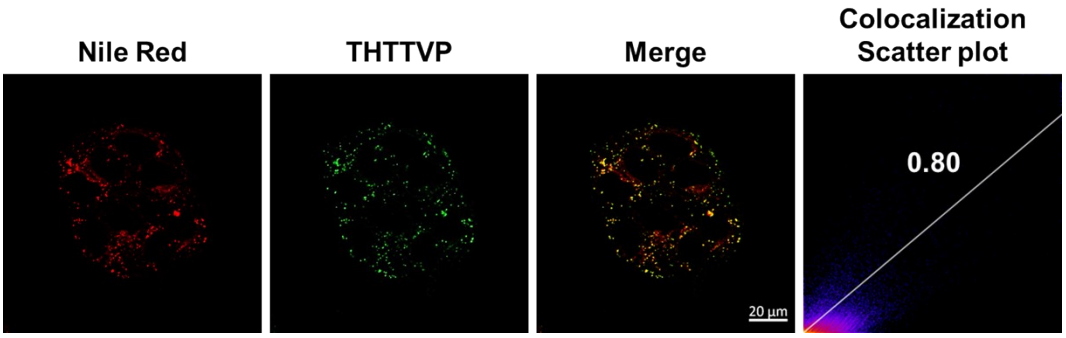


Figure S7. The colocalization images of Nile Red and THTTVP in the HeLa cells fixed by 4% paraformaldehyde. [THTTVP] = 5 μM; [Nile Red] = 0.5 μM.

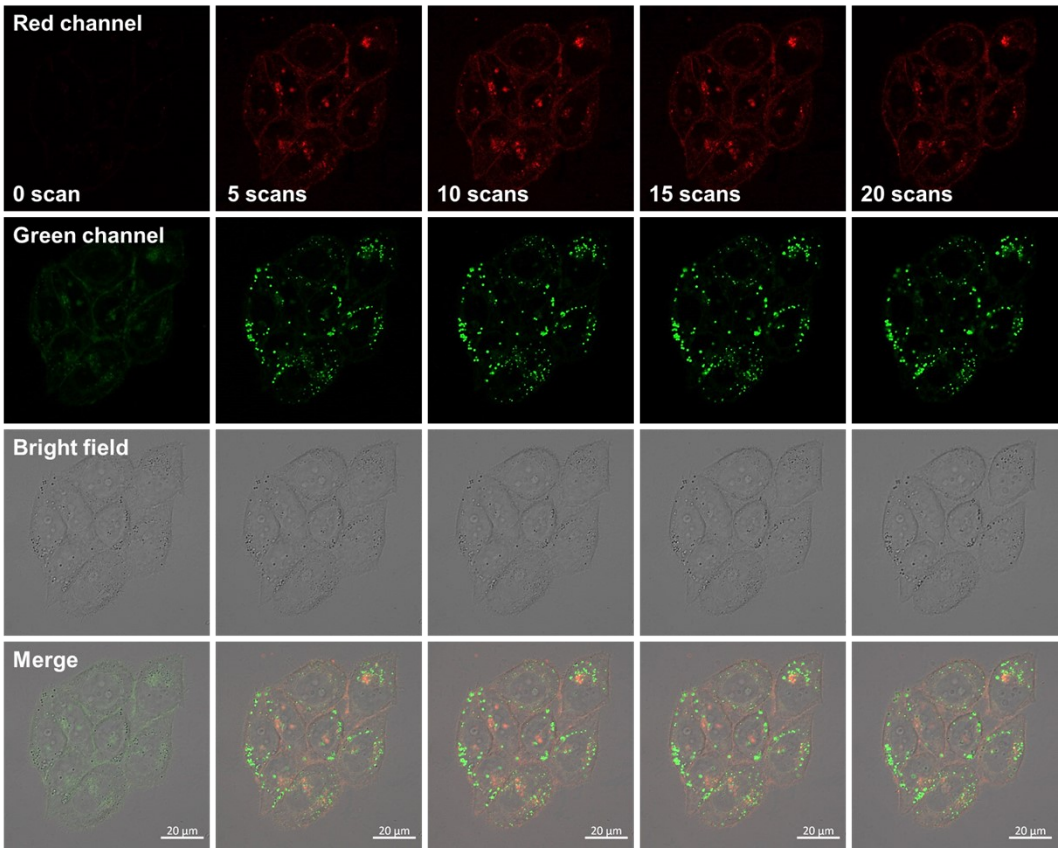


Figure S8. The photoactivatable images of HeLa cells treated with THTTVP after washing with fresh culture medium for three times. [THTTVP] = 5 μM. Laser power: 2%.

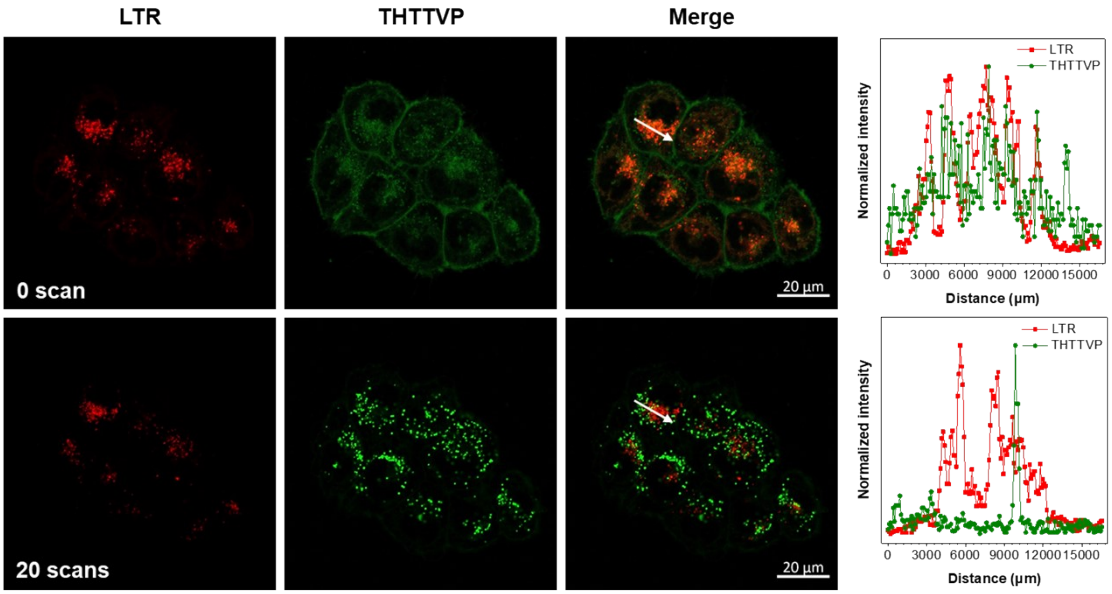


Figure S9. The colocalization images of LysoTracker Red (LTR) and THTTVP in the HeLa cells and the fluorescence profiles of the white arrow. [THTTVP] = 5 μM; [LTR] = 200 nM. For LTR channel, λ_{ex} = 543 nm, λ_{em} = 550−750 nm; For THTTVP channel, λ_{ex} = 405 nm, λ_{em} = 450−500 nm.

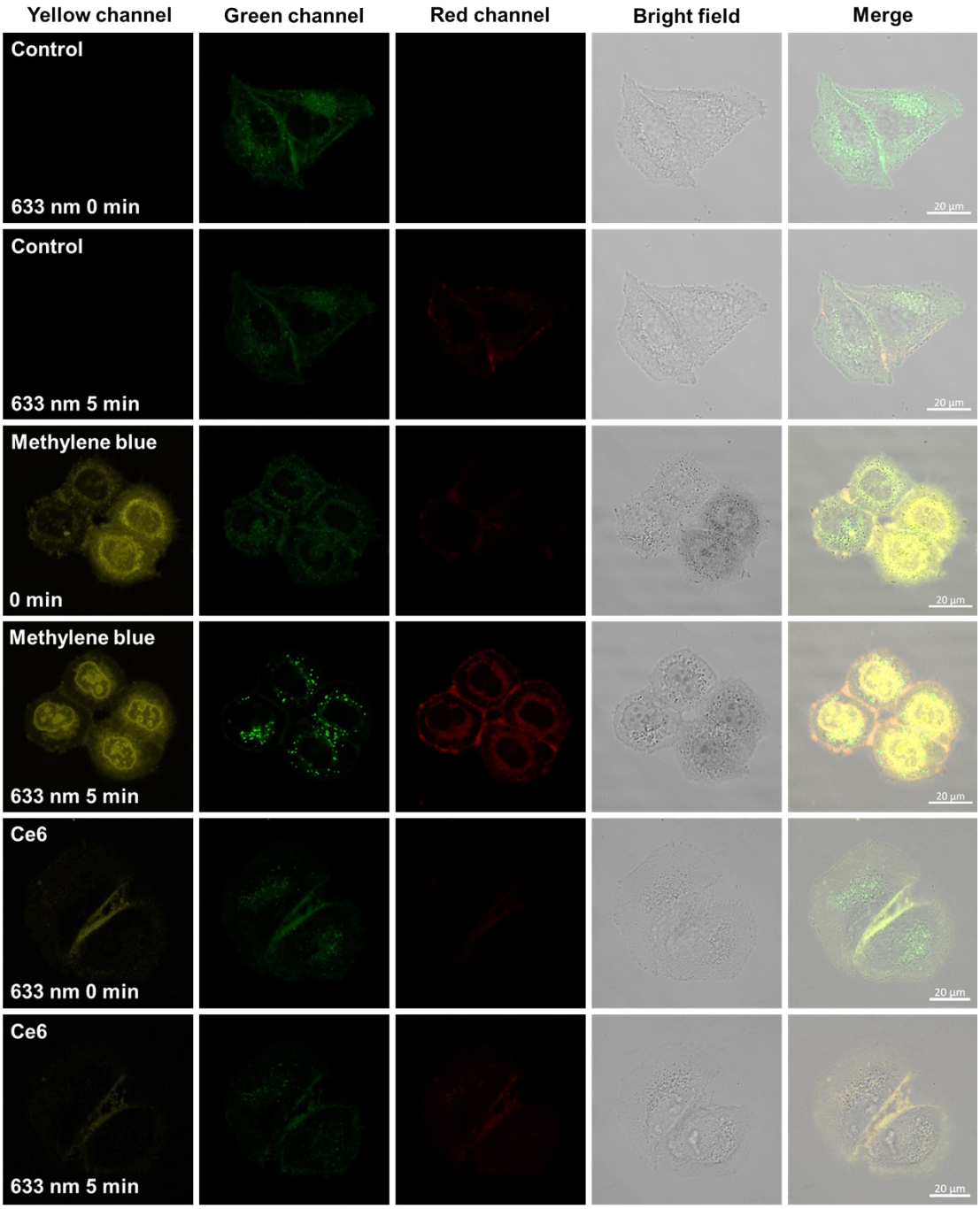


Figure S10. Turn-on monitoring of lysosomal escape induced by commercial photosensitizers (yellow) with THTTVP (green). [Methylene blue] = [Ce6] = 10 μM; [THTTVP] = 5 μM. Laser power: 2%.

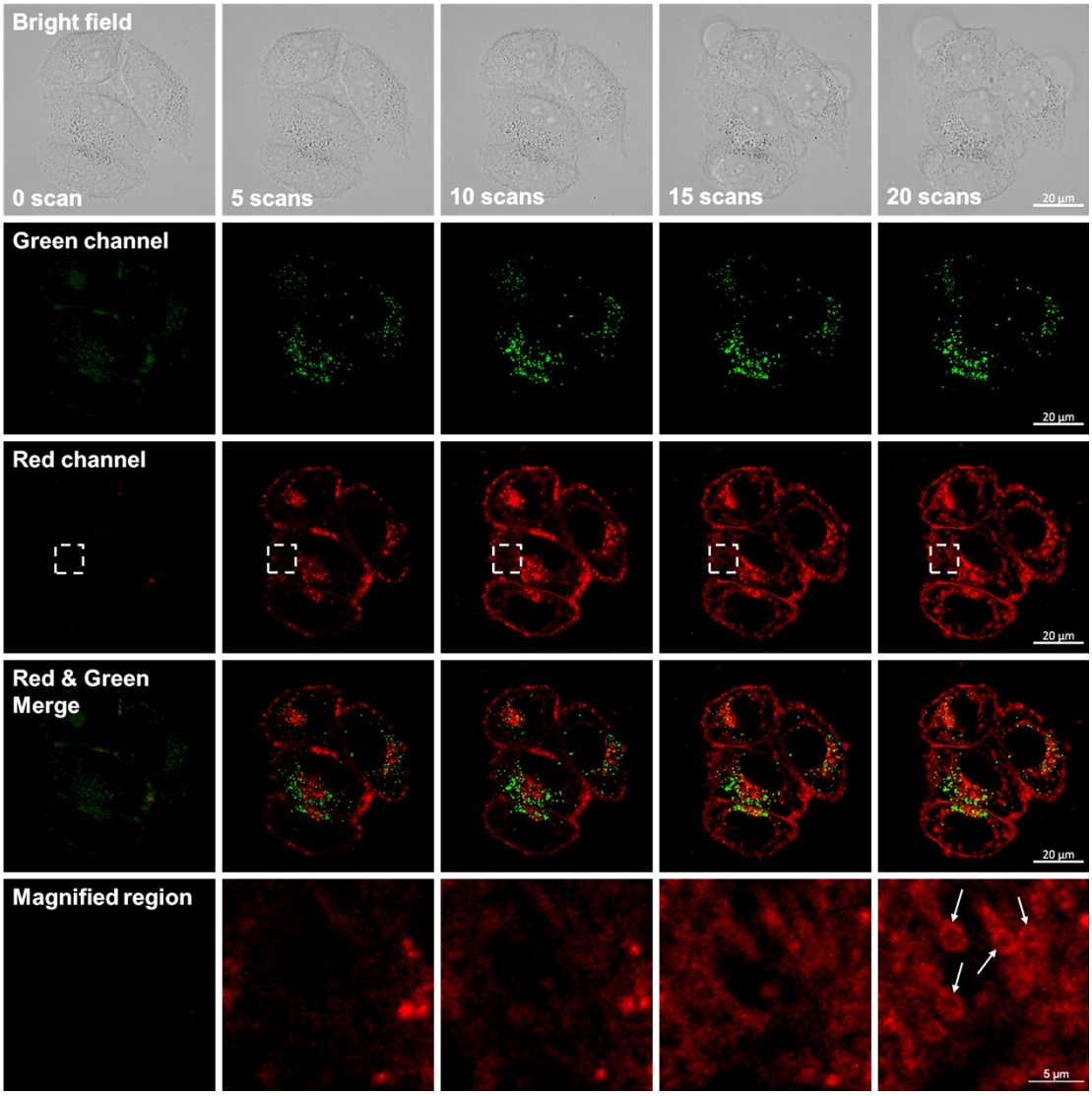


Figure S11. The photoactivatable images of cells stained with THTTVP after different 405 nm laser scanning numbers. Magnified region is amplified image from the marked area in the red channel image. The white arrows point to the ROS-induced mitochondrial vacuolization under light irradiation. [THTTVP] = 5 μM. Laser power: 2%.

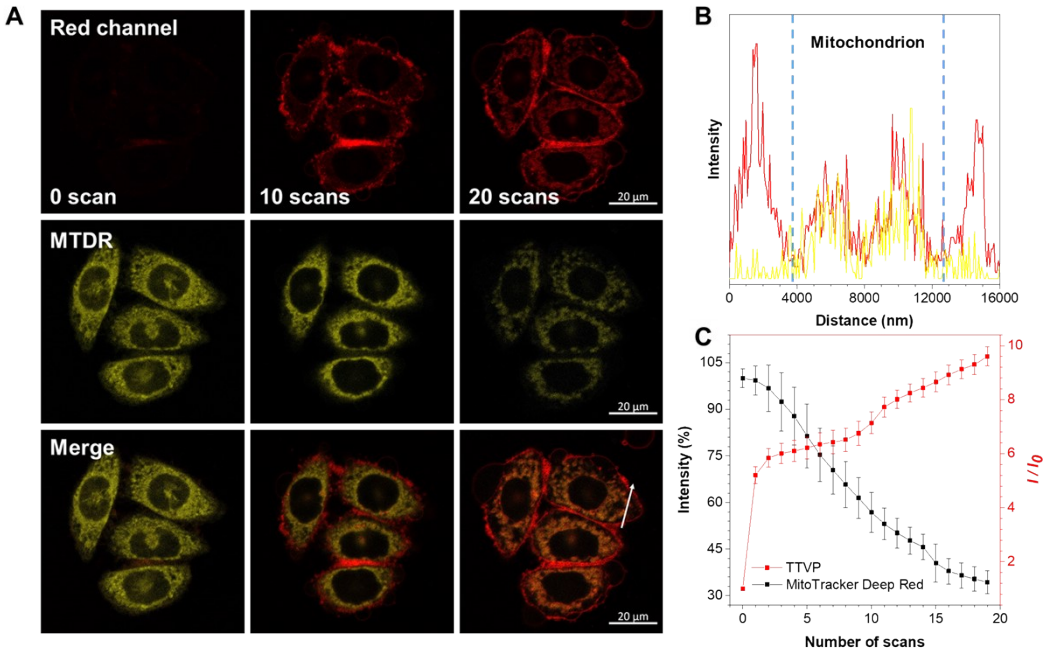


Figure S12. (A) The colocalization images of MitoTracker Deep Red (MTDR) (yellow) and the in situ generated TTVP (red). (B) The intensity profile of ROI line. (C) The fluorescence intensity changes of MitoTracker Deep Red and the in situ generated TTVP under continuous laser scanning. [THTTVP] = 5 μM; [MTDR] = 200 nM. Laser power: 2%.

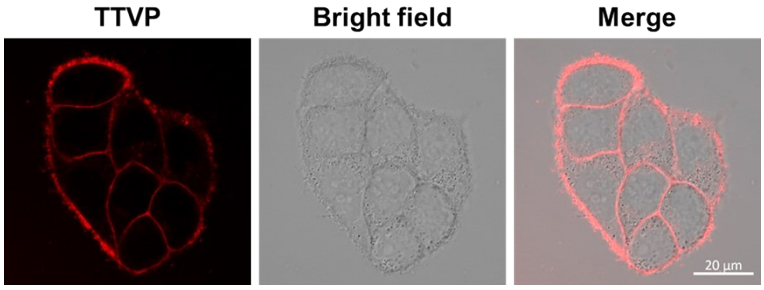


Figure S13. The CLSM images of cells stained with TTVP. [TTVP] = 10 μM.

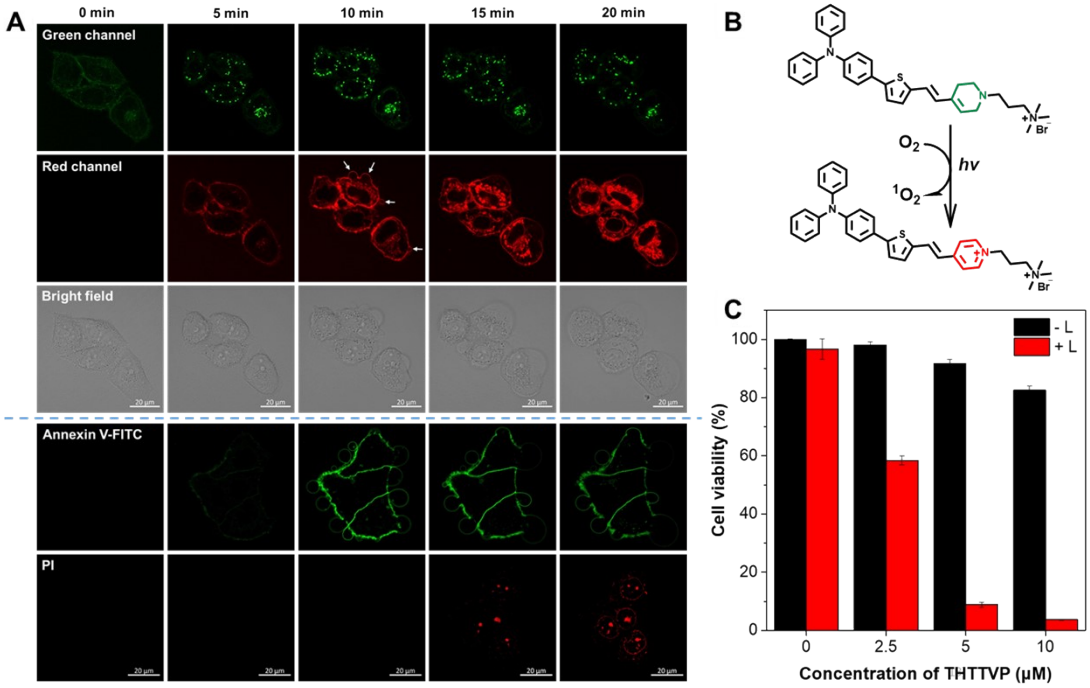


Figure S14. (A) Monitoring of apoptosis induced by THTTVP with apoptosis assay kit Annexin V-FITC/PI. (B) Photoactivation of THTTVP. (C) Cell viability of HeLa cells treated with different concentrations of THTTVP in the absence (− L) or presence (+ L) of white light irradiation (40 mW/cm^2).

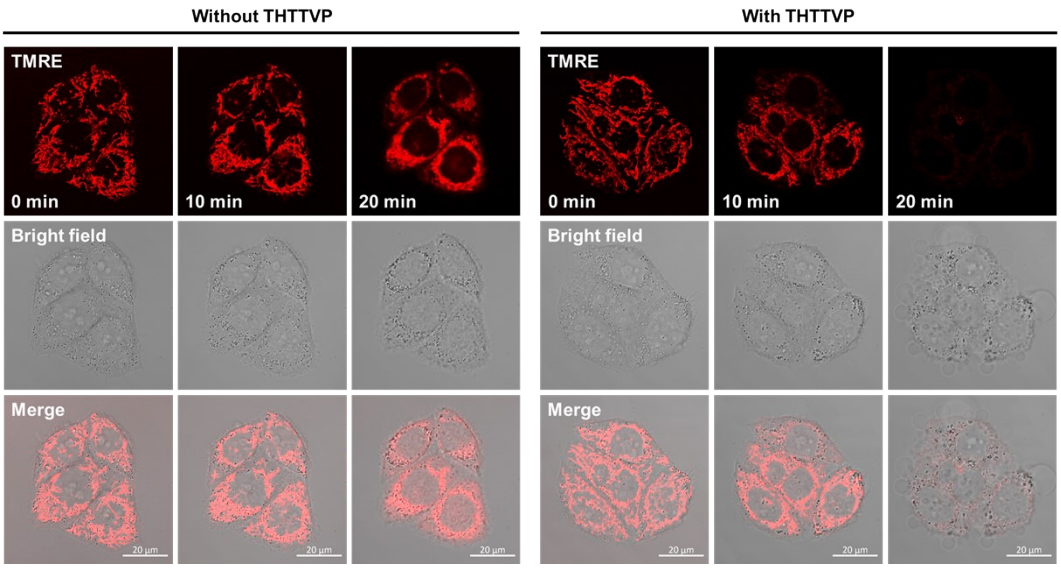


Figure S15. The fluorescence images of TMRE in HeLa cells stained with or without THTTVP under white light irradiation (40 mW/cm^2) for different time. [TMRE] = 100 nM; [THTTVP] = 5 μM.

Reference

1. Y. Xu, C. Wang, G. Ran, D. Chen, Q. Pang, Q. Song, Phosphate-Assisted Transformation of Methylene Blue to Red-Emissive Carbon Dots with Enhanced Singlet Oxygen Generation for Photodynamic Therapy. *ACS Appl. Nano Mater.* 2021, **4**, 4820−4828