Supporting Information

Cucurbit[7]uril-Anchored Polymer Vesicles Enhance Photosensitization in the Nucleus

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Experimental Section

1. Materials

5,10,15,20-Tetra(4-pyridyl)porphyrin, 3-Bromopropionic acid and Acrylic acid were purchased from TCI. 1-(4,5-Dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT) bought from Solarbio. Reactive Oxygen Species Assay Kit was obtained for Sigma . Singlet Oxygen Sensor Green was obtained for Invitrogen. Annexin V-FITC Apoptosis Detection Kit obtained for from BD. Q[7] was synthesized according to literature.[1]

2. Sample synthesis

2.1 Synthesis of TPPOR

5,10,15,20-Tetra(4-pyridyl)porphyrin (125 mg) and 3-bromopropionic acid (250 mg) were dissolved in DMF. The solution was stirred at 120 ℃ for 6 h in a nitrogen environment. The solution was allowed to cool and was filtered. The solid precipitate was washed with trichloromethane and diethyl ether thoroughly then dried under vacuum. The yield was 62 %.

2.2 Synthesis of Q[7]-PAA

Q[7]-PAA was synthesized by dissolving of Q[7] (250 mg, 0.18 mmol) and ammonium persulfate (91.2 mg, 0.4 mmol) in distilled water (25 mL).[2] The solution was stirred at 75 ℃ in a water bath for 2 h, with N_2 was bubbled through the solution. After then, a 0.4 mol/L aqueous solution of acrylic acid (50 mL, 2 mmol) was added by drop-wise and stirred for 6h. The solution was allowed to cool and was filtered. The filtrate was purified by dialysis (MWCO 2000) against water for 24 h to yield a polymer solution, and then concentrated to a volume of 2 mL on a rotary evaporator. The solution was added dropwise into acetone The solid precipitate was filtered and washed with acetone thoroughly then dried under vacuum at 40 ℃ for 48 h. The yield was ~45%.

3. Singlet Oxygen Generation Experiments

The degree of singlet oxygen generated in solution. SOSG was dissolved in methanol to prepare a 5 mM solution and was prepared fresh at the start of each experiment. TPPOR, TPPOR/$(Q[7])_4$ or TPPOR/Q[7]-PAA (C_{TPPOR} = 10.0 μM) was mixed with SOSG (5 μM) in 10 mM sodium phosphate buffer (pH = 7.2). Samples were irradiated under laser (300 mW/cm^2) at 640 nm. At 30 s intervals, SOSG fluorescence was measured by exciting at 495 nm and collecting between 505 and 600 nm.

4.Cell culture and cytotoxicity determination

4.1 Cell culture

Cell line SH-SY5Y (Human Neuroblastoma Cancer) was purchased from American Tissue Culture Collection (ATCC). SH-SY5Y cells were cultured in DMED /F12 (Hyclone) medium supplemented with 10% FBS (fetal bovine serum, Gibco) and 1% penicillin/streptomycin (Hyclone). All cells were incubated at 37 ℃ with 5% CO_2.

4.2 The cellular localization

Cells were seeded in confocal dishes (Φ 15 mm glass bottom) and cultured overnight for attachment. Then, cells were incubated with TPPOR, TPPOR/$(Q[7])_4$ or TPPOR/Q[7]-PAA (C_{TPPOR} = 10.0 μM) for 24 h. After removed the medium and washed three times with PBS, cells were incubated with DAPI (Solarbio, Beijing) for 30min. Then, cells were washed with PBS for five times and imaged with a confocal laser-scanning microscope. The fluorescence of TPPOR, TPPOR/$(Q[7])_4$ or TPPOR/Q[7]-PAA was excited by a 546 nm laser, and the fluorescence of DAPI was excited by 488 nm laser. The same cells, which were incubated with TPPOR, TPPOR/$(Q[7])_4$ or TPPOR/Q[7]-PAA, were irradiated under laser beam (300 mW/cm^2) at 640 nm for 180 s, and then were incubated for 8 h to observe morphology change by the same way.

4.3 ROS detection

ROS was measured by a reactive Oxygen Assay Kit using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) as the the fluorescence probe. For ROS measurements in cells : cells were seeded on 24-well plates (10^5 cells/well) and cultured in 5% CO_2 atmosphere at 37 ℃; after cultured overnight, cells were incubated with TPPOR, TPPOR/$(Q[7])_4$ or TPPOR/Q[7]-PAA (C_{TPPOR} = 10.0 μM); After being cultured 24 h, cells were washed three times and incubated with DCFH (10 μM) for 30 min. And then, cells were irradiated with a 525 nm laser on different time (1 min, 2 min, 3 min) and the fluorescence images well collected with excitation at 488 nm and emission at 525 nm. Mean density = (IOD SUM)/(area sum), Analysis by Image-Pro plus 6.0 Image Software.

4.4 Cell apoptosis assay

SH-SY5Y cells were seeded in a 6-well plate at a density of 4×10^5 cells per well and incubated with TPPOR, TPPOR/$(Q[7])_4$ or TPPOR/Q[7]-PAA (C_{TPPOR} = 10.0 μM) for 24 h. After replacing the media with fresh media, the cells were irradiated under laser (300 mW/cm^2) for 3 min and then further incubated for 8 h. Then the cells

were double-stained with 100 μL of buffer containing FITC-labeled annexin V and PI for 15 min at room temperature. Measured with NovoCyte. In Vitro Cytotoxicity Assay. SH-SY5Y cells were seeded in 96-well plates (4×10^4 cells/well) and cultured overnight for attachment.

4.5 Dark-toxicity study

TPPOR, TPPOR/$(Q[7])_4$ or TPPOR/Q[7]-PAA were added into cells at the TPPOR concentration of 1, 5, 10, 50, μM respectively for 24 h. The media were replaced with fresh medium (without FBS and penicillin/streptomycin) after the cells were washed three times with phosphate-buffered saline (PBS). After further incubation for 24 h, the viability of cells was measured with MTT assay.

4.6 Phototoxicity study

Cells were firstly incubated with TPPOR, TPPOR/$(Q[7])_4$ or TPPOR/Q[7]-PAA at the different concentration of TPOR (C_{TPPOR} = 1, 2.5, 5, 7.5, 10 μM) for 24 h. After replacing the media with fresh media, the cells were irradiated under the different laser beam (50, 100, 150, 300 mW/cm^2) at 640 nm for 0, 30, 60, 90, 120, 150, 180 s respectively. The cells were continually cultured for additional 24 h, the viability of cells was measured with MTT assay.

All experiments were performed at least 3 times. Analysis was performed using GraphPad Prism 7. The one-way ANOVA test was used to compare the differences between multi groups and $p < 0.05$ was considered statistically significant.

Reference

1. A. Day, A. P. Arnold, R. J. Blanch and B. Snushall, *J. Org. Chem.*, 2001, **66**, 8094
2. R. H. Gao, Y. Fan, B. Xiao, P. Chen, J. X. Zhang, Q.-D. Zhou, S. F. Xue, Q. J. Zhu and Z. Tao, *RSC Adv.*, 2015, **5**, 65775

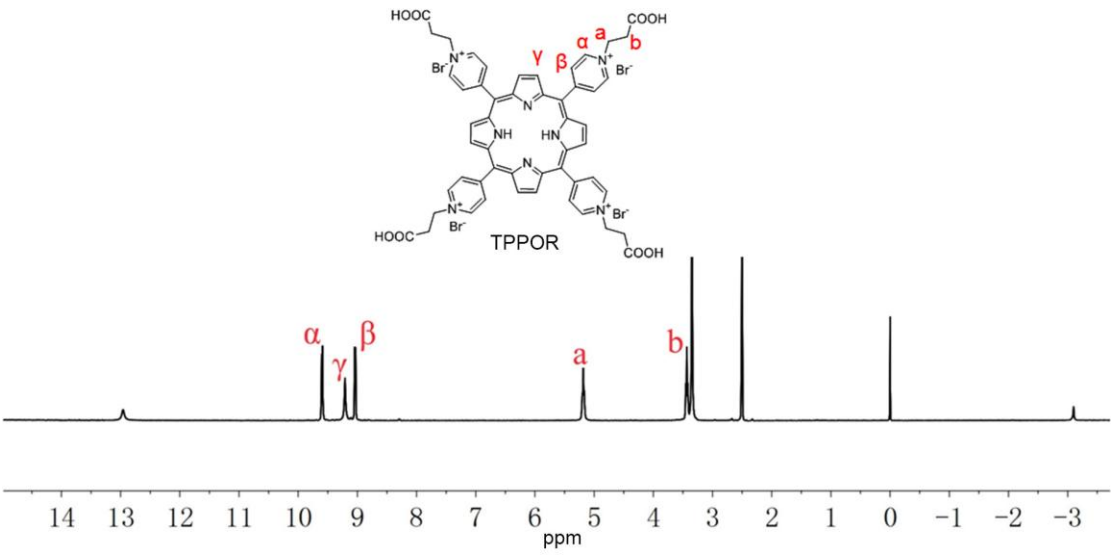


Fig. S1 ^{1}H NMR spectra (400 MHz, d_6-DMSO) of the porphyrin cationic guest (TPPOR).

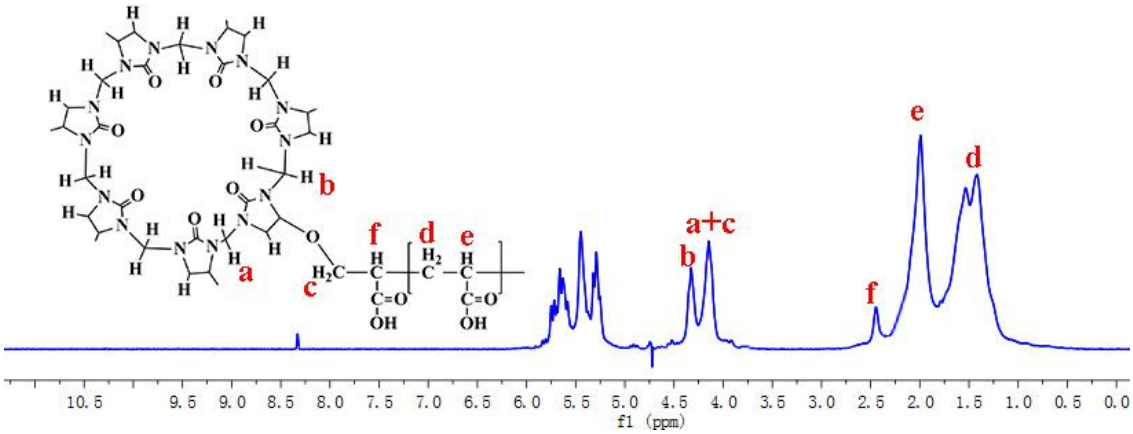


Fig. S2 ^{1}H NMR spectra (400 MHz, D_2O) of Q[7]-PAA.

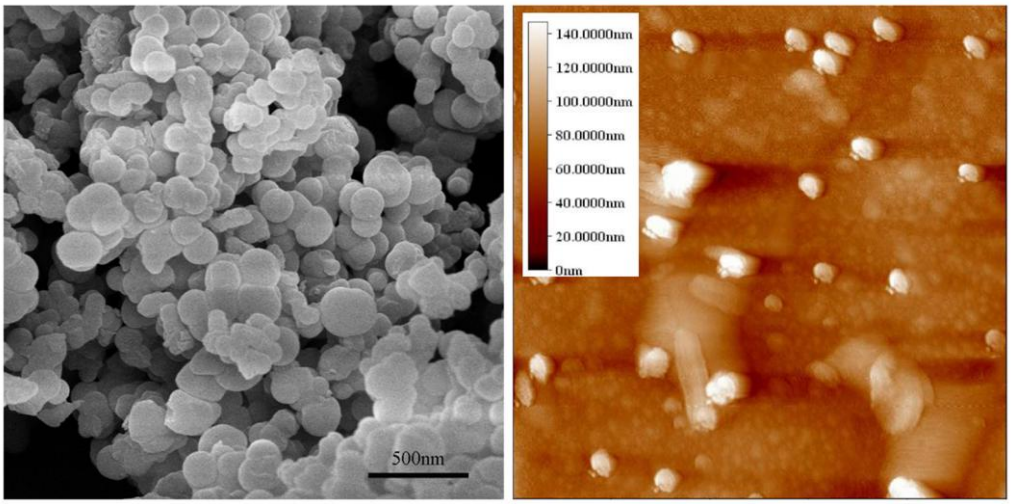


Fig. S3 (a) SEM and (b) AFM images of Q[7]-PAA based spherical particles in aqueous solution.

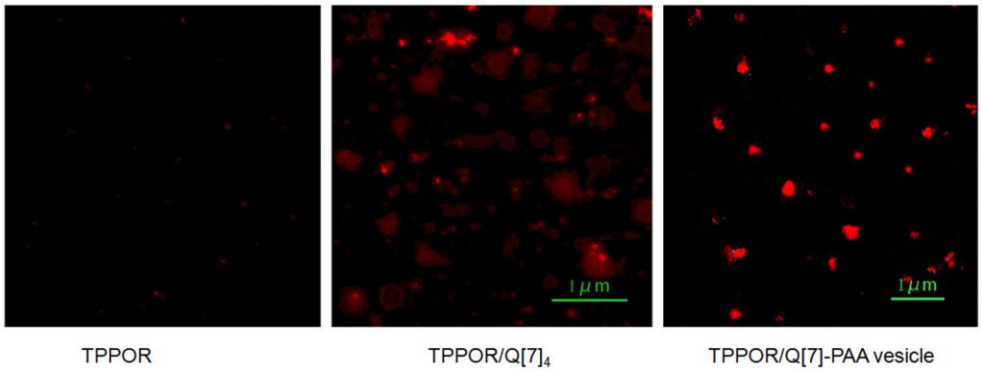


Fig. S4 CLSM images of TPPOR, TPPOR/Q[7]$_4$, and TPPOR/Q[7]-PAA vesicles in aqueous solution (C_{TPPOR} = 10.0 μM).

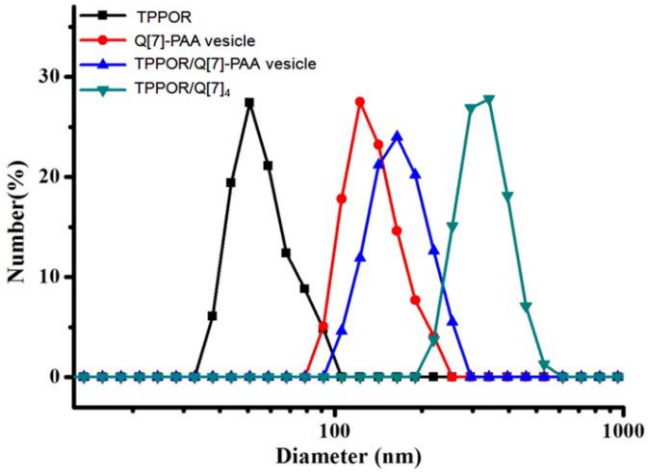


Fig. S5 Dynamic light scattering results of TPPOR, Q[7]-PAA and their mixture in water. The data represent the hydrodynamic diameters (DH) in water (PH 7.2) at 25 ℃. The concentration of TPPOR is 10.0 μM.

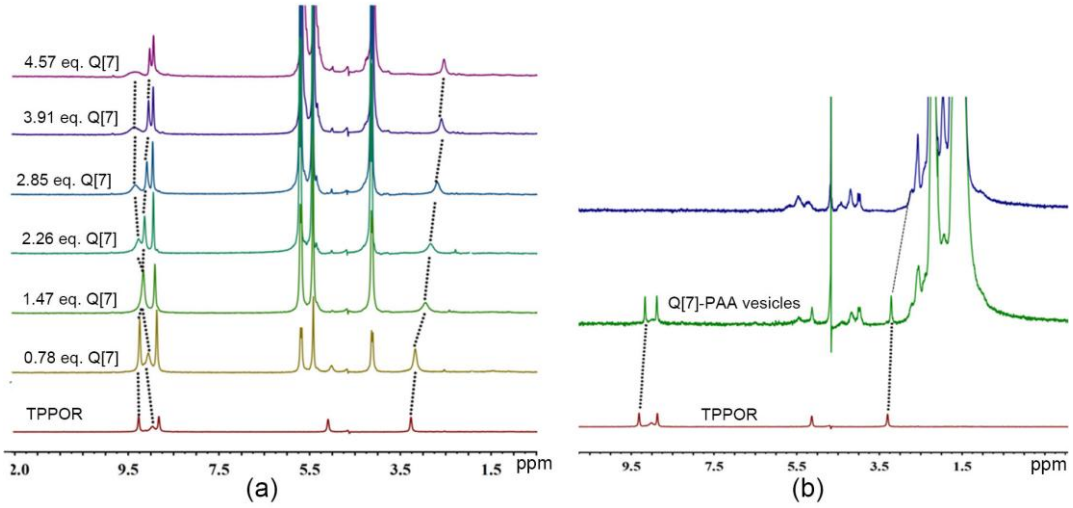
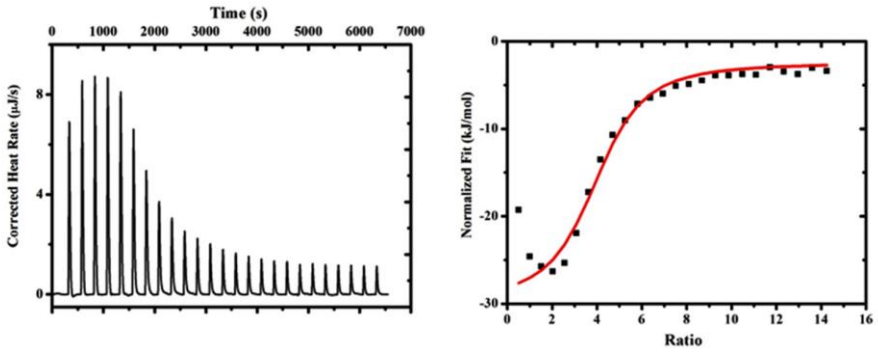


Fig. S6 ^{1}H NMR spectra of TPPOR (1.0 mmol, D_2O, pD=7.2) in the presence of (a) single Q[7] host and (b) Q[7]-PAA vesicles.



$K(M^{-1})$	ΔH°（kJ·mol^{-1}）	TΔS°（kJ·mol^{-1}）	n	Model
1.62(±0.23)×10^6	-27.34(±1.13)	2.384	4.019(±0.026)	Independent

Fig. S7 ITC data for the titration of Q[7] with TPPOR in water (pH 7.2), the "molar ratio" is defined as TPPOR:Q[7].

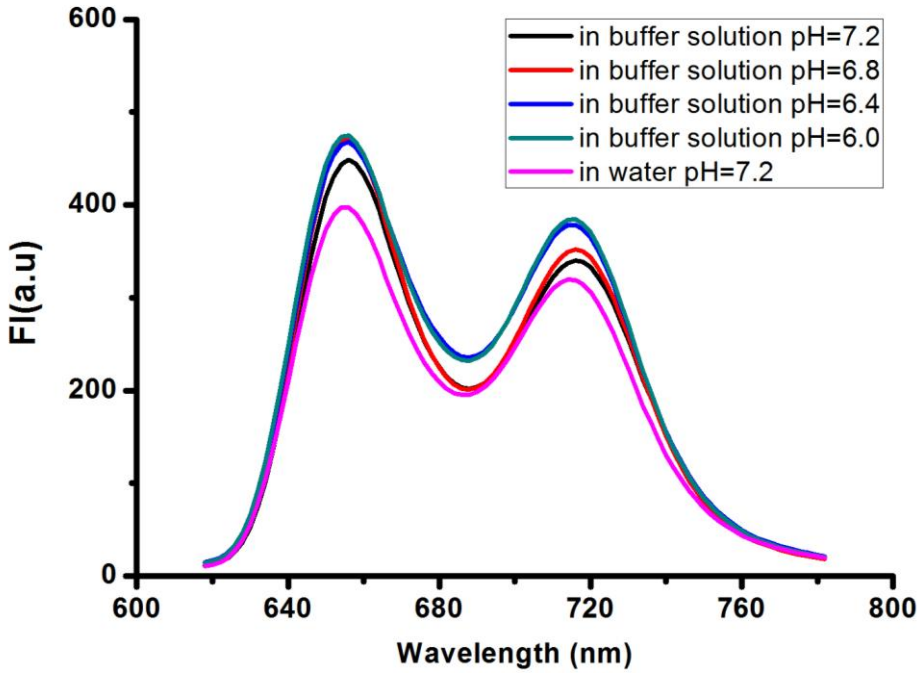


Fig. S8 Fluorescence spectra of TPPOR (10.0 μM) in the presence of Q[7]-PAA vesicles in 10 mM $H_2PO_3^-$-HPO_3^{2-} buffer saline solution

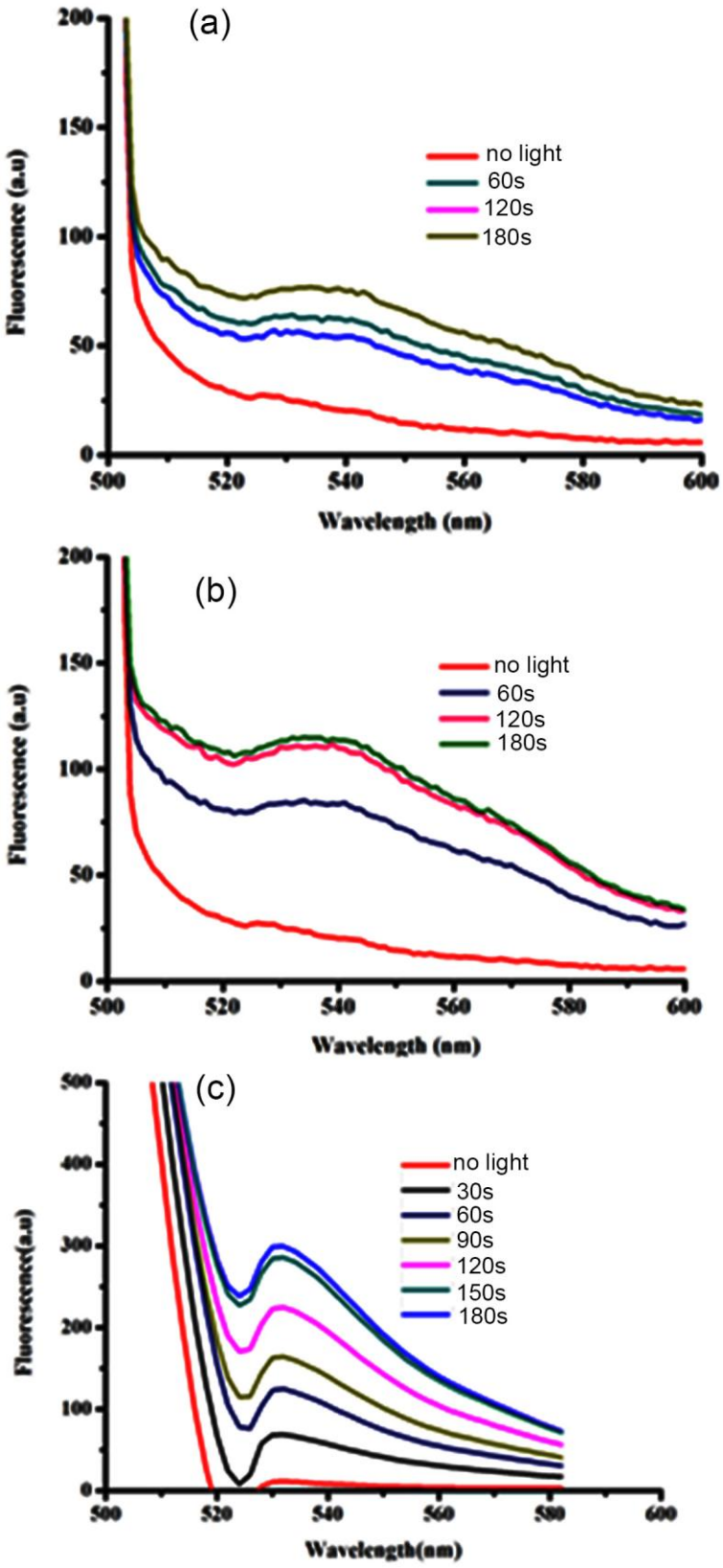


Fig. S9 Fluorescence intensity changes of SOSG in the presence of (a) TPPOR, (b) TPPOR/Q[7]$_4$, (c) TPPOR/Q[7]-PAA vesicle (concentration of TPPOR was fixed to 10.0 μM) and after irradiation with a 640 nm laser beam (300 mW) for various time periods.

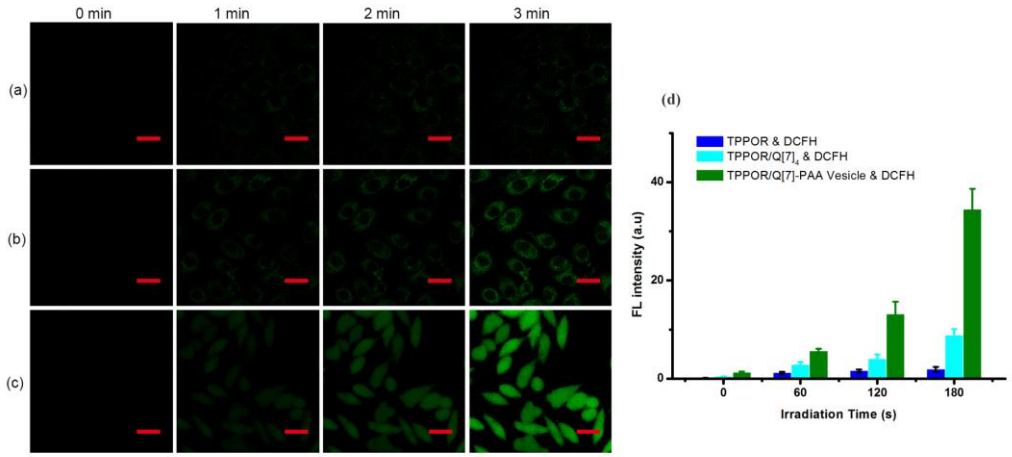


Fig. S10 Detection of ROS generation in the excited state under light irradiation in SH-SY5Y cells in the presence DCF-DA and treated with; (a) TPPOR, (b) TPPOR/Q[7]$_4$, (c) TPPOR/Q[7]-PAA, respectively (C_{TPPOR} = 10.0 μM), (d) the related fluorescence intensity changes. The scale bar represents 20 μm

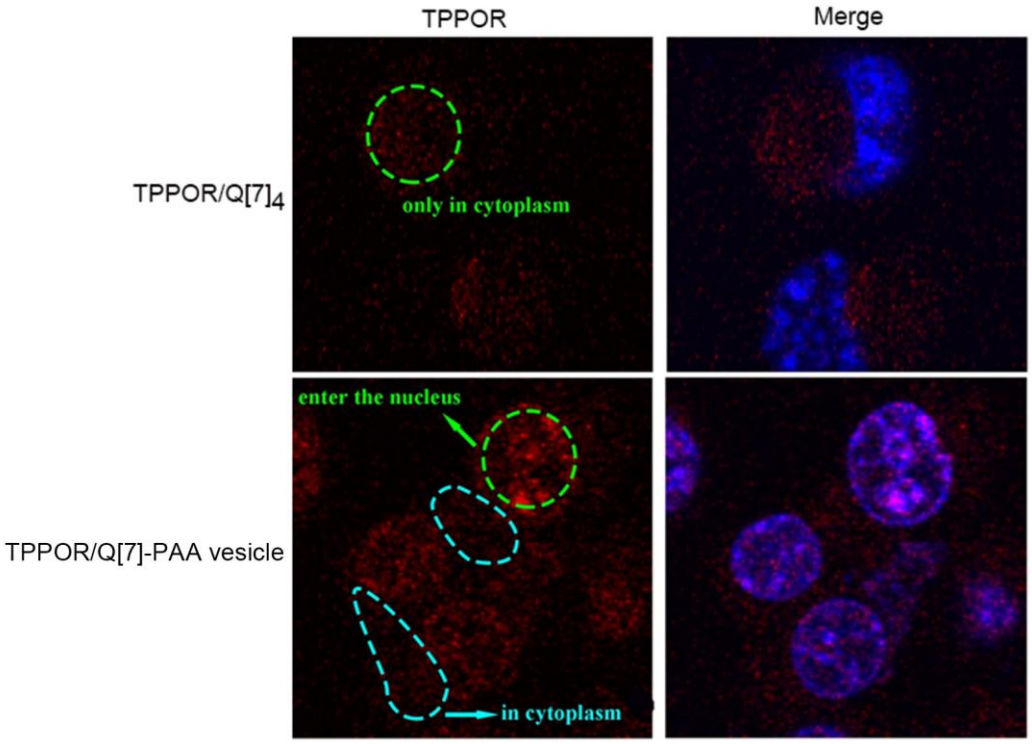


Fig. S11 CLSM images of SH-SY5Y cells recorded after 3 min irradiation and 8 h co-incubation with TPPOR/Q[7]$_4$, and TPPOR/Q[7]-PAA vesicle (C_{TPPOR} = 10.0 μM). The cell nucleus was stained with DAPI.

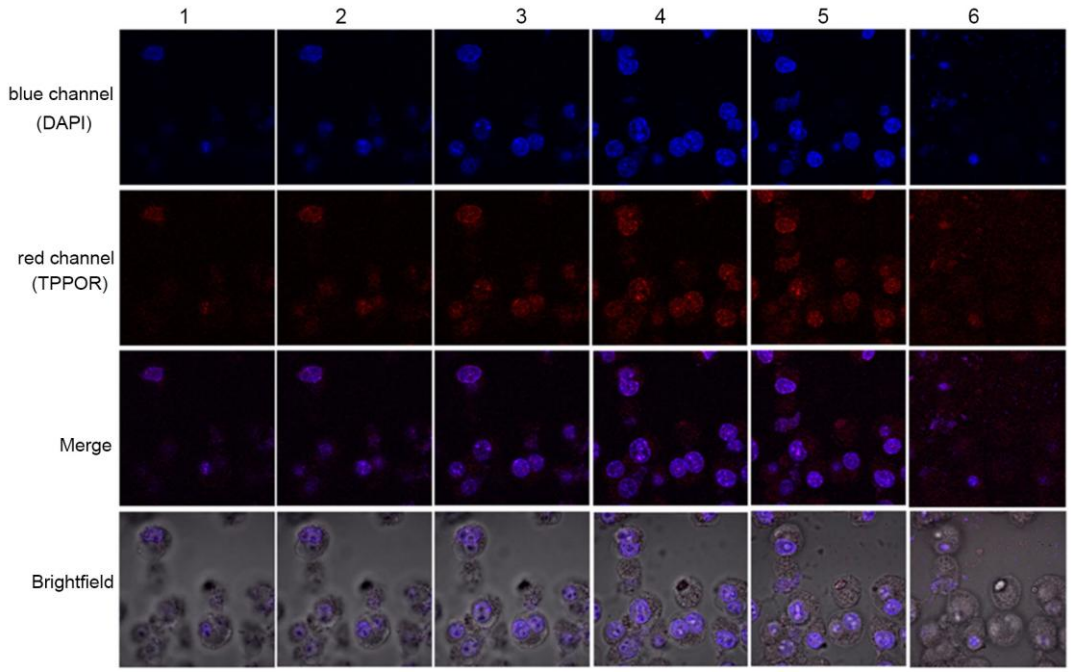


Fig. S12 Sectional CLSM images along z-axis for TPPOR/Q[7]-PAA vesicles internalized SH-SY5Y cells. The red fluorescence is obtained from the TPPOR/Q[7]-PAA vesicles. The cell nucleus was stained with DAPI.

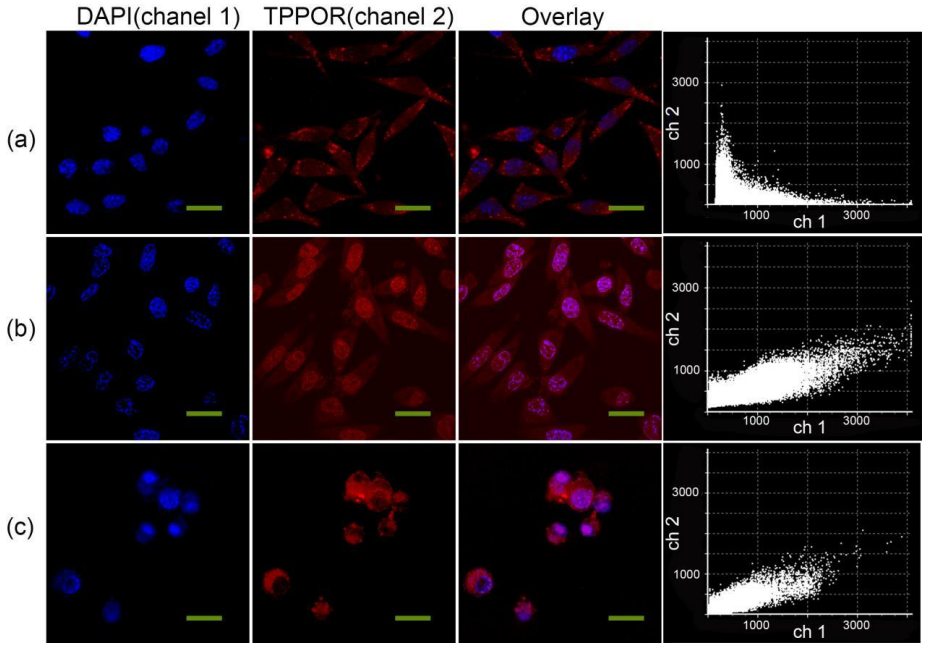


Fig. S13. Cellular internalization of TPPOR/Q[7]-PAA vesicles were conducted on SH-SY5Y cells and co-incubation in different conditions and times: (a) 24 h, (b) after 3 min irradiation and 1 h, (c) after 3 min irradiation and 4 h.

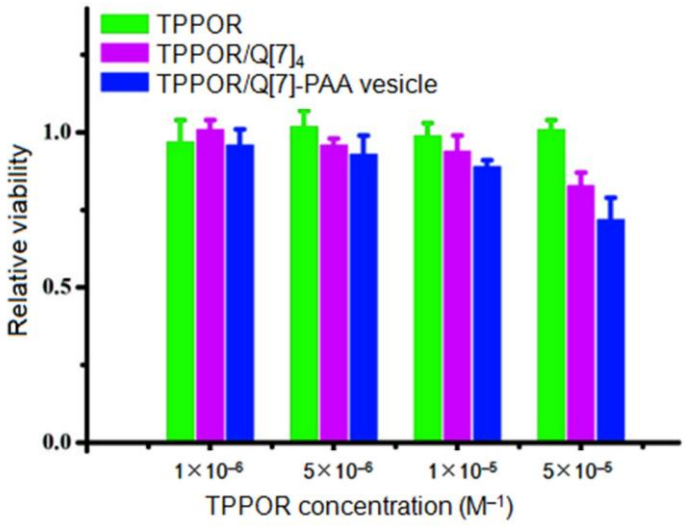


Fig. S14 Percentage of SH-SY5Y cells viability remaining after cell treatment with different concentrations of TPPOR, TPPOR/Q[7]$_4$, TPPOR/Q[7]-PAA vesicles, respectively (untreated cells were considered to have 100% survival). Cell viability was assayed by the MTT method (values: mean ± standard deviation).

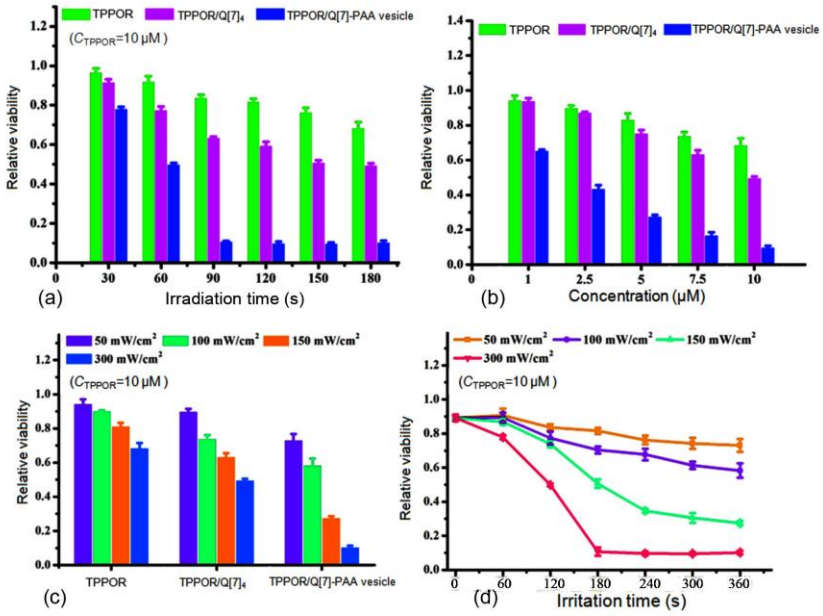


Fig. S15 Percentage of SH-SY5Y cells viability remaining after cell treatment with TPPOR, TPPOR/Q[7]$_4$, TPPOR/Q[7]-PAA vesicle and irradiation with 640 nm laser beam: (a) irradiation (300 mW) with different time; (b) different concentrations under light irradiation (300 mW) 180 s; (c-d) irradiation with different brightness. (untreated cells were considered to have 100% survival). Cell viability was assayed by the MTT method (values: mean ± standard deviation).

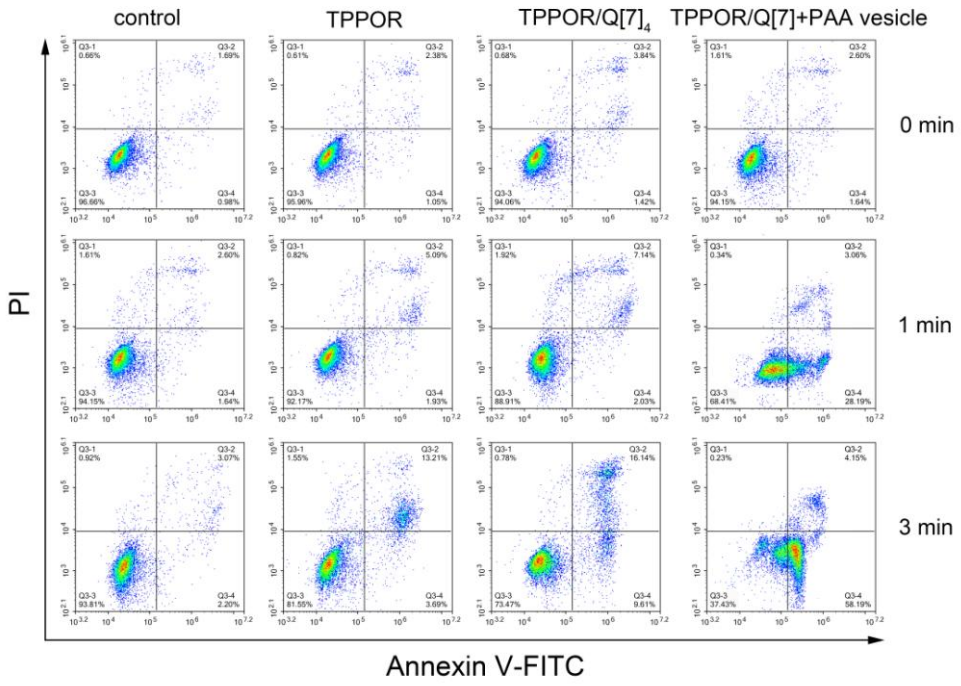


Fig. S16 The cell death and apoptosis of SH-SY5Y cells after staining with annexin V–FITC and propidium iodide (PI) by flow cytometry. Untreated SH-SY5Y cells were used as a control. Cells that were negative for both annexin V–FITC and PI staining were classified as alive, whereas cells stained positively for annexin V–FITC and negatively for PI were classified as apoptotic. Cells that stained positively for PI were classified as necrotic.