Electronic Supporting Information for

A Novel pH-Activated AIEgen Probe for Dynamic Lysosome Tracking and High-Efficiency Photodynamic Therapy

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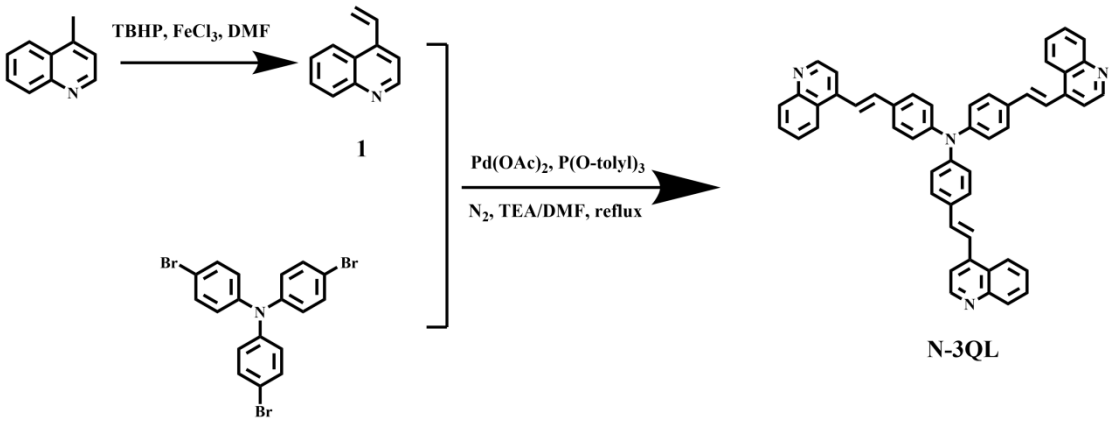
Reagents and Equipment

All chemical and biological reagents are purchased from local suppliers. Mass spectrometry uses matrix-assisted laser dissociation time of flight mass spectrometry imager (Bruker, Germany). NMR spectra were recorded on a Bruker 400MHz Advance spectrometer (Bruker, Germany) using TMS as an internal standard. The molar absorption coefficient was measured by Hitachi U-4100 UV absorption spectrometer (Hitachi, Kyoto, Japan). The fluorescent images of cells were obtained Nikon confocal two-photon microscope (Nikon, Japan).

Synthesis of N-3QL



Scheme S1. Schematic illustration of the synthesis of N-3QL.

Compound 1 [1] (155 mg, 1 mmol) and tri(4-bromobenzene) amine (119.7mg, 0.25 mmol) were weighed in a round-bottom flask. Catalytic amounts of $Pd(OAc)_2$ and tri(o-methylphenyl) phosphorus were added in the atmosphere of N_2, and 10 mL triethylamine mixed with DMF (V/V = 2/1) were added. Reflux was performed at 80℃ for 8 h. After the reaction, the organic phase was washed with saturated sodium chloride solution, and the solvent was reduced to vacuum evaporation. The crude product was purified by silica gel column chromatography to obtain yellow solid product N-3QL (158.4 mg, 0.225 mmol) with a yield of 90%. 1H NMR (400 MHz, DMSO-d6) δ 8.91 (d, J = 4.8 Hz, 1H), 8.56 (d, J = 8.6 Hz, 1H), 8.08 (d, J = 3.8 Hz, 1H), 8.05 (d, J = 4.0 Hz, 1H), 7.89 (dd, J = 9.2, 6.6 Hz, 3H), 7.82 (t, J = 7.7 Hz, 1H), 7.70 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 16.5 Hz,

1H), 7.20 (d, J = 8.3 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-d) δ 150.16, 148.69, 147.41, 143.08, 134.35, 131.86, 130.12, 129.39, 128.37, 126.52, 126.40, 124.49, 123.43, 121.67, 116.82. MALDI-TOF: m/z $[M + H]^+$: 705.294; found: 705.303.

Preparation of Britton-Robinson Buffer Solution

Different volumes of sodium hydroxide (0.2 M) were added to a mixture of phosphoric acid, boric acid and acetic acid at a concentration of 40 mM and corrected with a pH meter to obtain different pH values of Britton-Robinson (BR) buffer. A series of pH buffers including 3.00, 3.37, 3.72, 4.00, 4.31, 4.61, 5.00, 5.40, 5.64, 6.00, 6.38, 6.66, 7.00, 7.36, 7.73 and 8.00.

Investigation of Optical and Photosensitive Properties

10 μL N-3QL (1 mM, DMSO) was mixed with 990 μL BR buffer solution of different pH values, and then added to 1 mL quartz cuvette to measure the UV absorption and fluorescence emission spectra.

N-3QL (10 μM) was mixed with BR buffer solutions containing ABDA (50 μM) at different pH values, respectively, and irradiated with white light (50 mW · cm^{-2}). UV absorption of the solution was measured by Hitachi U-4100 every 10 s. The absorbance at 378 nm was recorded.

Cell Culture and Fluorescence Imaging

HeLa and L02 cells cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37 °C in a moist atmosphere containing 5% CO_2. Cells with a density of 1 × 10^4 cells /mL were placed in a cell culture dish and incubated at 37 ^{0}C with 5% CO_2 for 24 h. Then, remove the medium and wash it with PBS for three times, add the medium containing 10 μM N-3QL and culture it at 37 °C for 1 h, then wash it with PBS for three times to remove the dye in the solution. Fluorescence images of the cells were obtained by using a Nikon A1R microscope (N-3QL：λ_{ex} = 405 nm, λ_{em} = 525-575 nm；N-3QL: λ_{ex} = 405 nm, λ_{em}

= 600-650 nm).

Measurement of Singlet Oxygen in Living Cells

HeLa cells with a density of 1×10^4 cells /mL were placed in a cell culture dish and incubated at 37°C with 5% CO_2 for 24 h. Before imaging, the cells were incubated with TPE-TThPy and DCFH-DA for 30 min, thereafter followed by white light irradiation (50 $mW \cdot cm^{-2}$) for 3 min. The dark group didn't do anything before imaging. DCFH-DA: λ_{ex} = 488 nm, λ_{em} = 500-550 nm.

Cell Cytotoxicity Assay

HeLa cells were cultured in 96-well plates, respectively. Each well was inoculated with a cell density of 1×10^5 cells /mL in 100 μL DMEM containing 10% FBS. After incubation for 24 h, the non-illumination group was incubated with 100 μL DMEM containing N-3QL (40, 30, 20, 10, 5, 2, 1, 0 μM). The light group was also incubated with 100 μL DMEM containing different concentrations of N-3QL (10, 7.5, 5.0, 2.5, 1.0, 0.50, 0.25, 0 μM). After 1h incubation, the group was irradiated with 5 $mW \cdot cm^{-2}$ white light for 15 min. Incubation was continued for 12 h. Thereafter, 10 μL of Cell Counting Kit-8 (CCK-8) solution was respectively added, then continue to incubate for 2 h, and measure the absorbance of each well solution with multi-functional microplate reader at 450 nm.

Flow Cytometric Assay

HeLa cells were cultured in 6-well plates. The cell density per well was 1×10^5 cells/mL and was seeded in 2 mL of DMEM. After 24 h incubation, each well was treated in different ways and divided into 4 groups (“PBS” group,”PBS + Light” group,”N-3QL” group and ”N-3QL + Light” group). After treatment, the cells were incubated for 8 h, and then rapidly digested with trypsin and washed with PBS for 3 times. Then, 100 μL of 1×Assay Buffer containing Annexin V-FITC and PI was added to the centrifuge tube. The Assay was incubated at 37°C for 30 min and detected by flow cytometry (Annexin V-FITC: λ_{ex} = 488 nm, λ_{em} = 500-550 nm; PI: λ_{ex} = 561 nm, λ_{em} = 590-640 nm).

Additional Figures

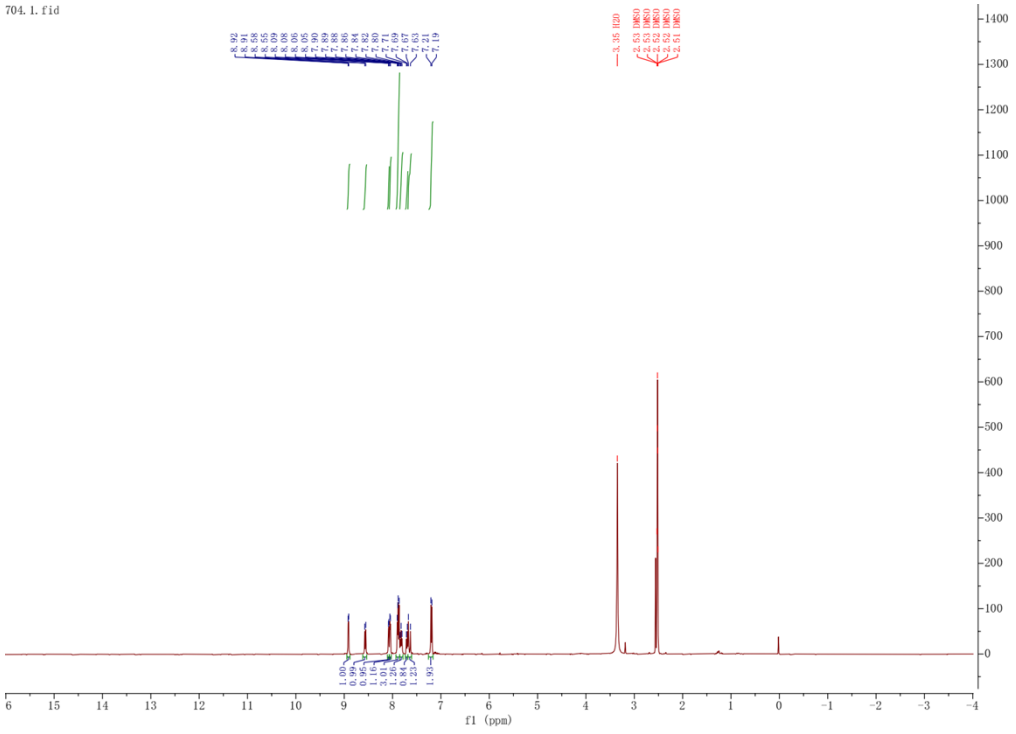


Figure. S1. ^{1}H NMR of N-3QL.

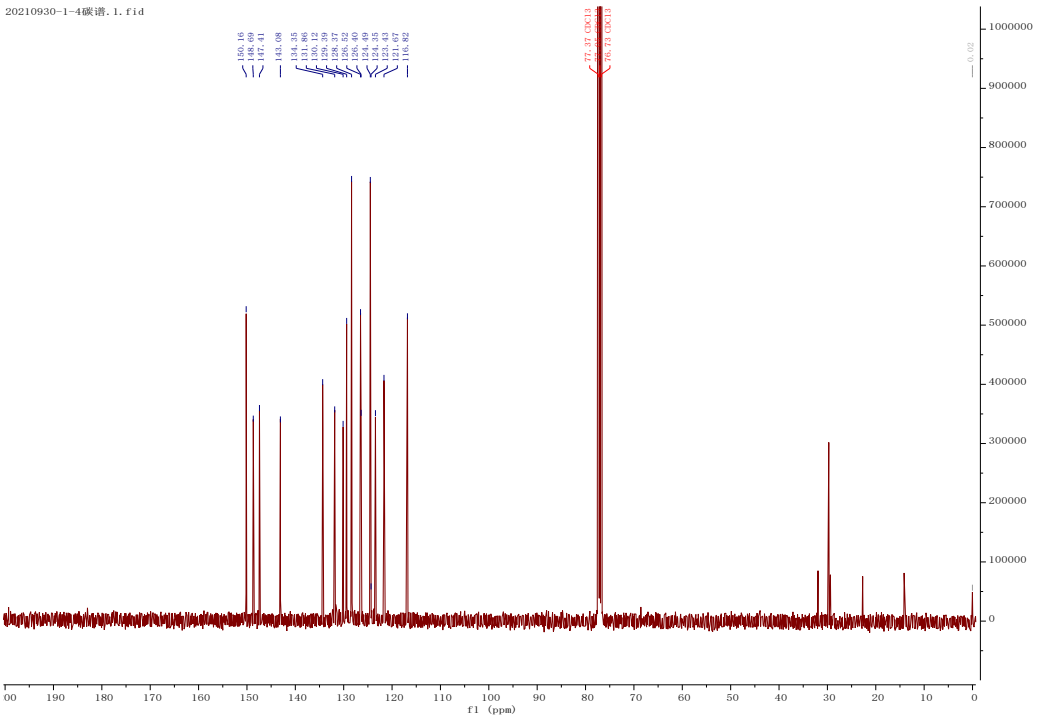


Fig. S2. ^{13}C NMR of N-3QL

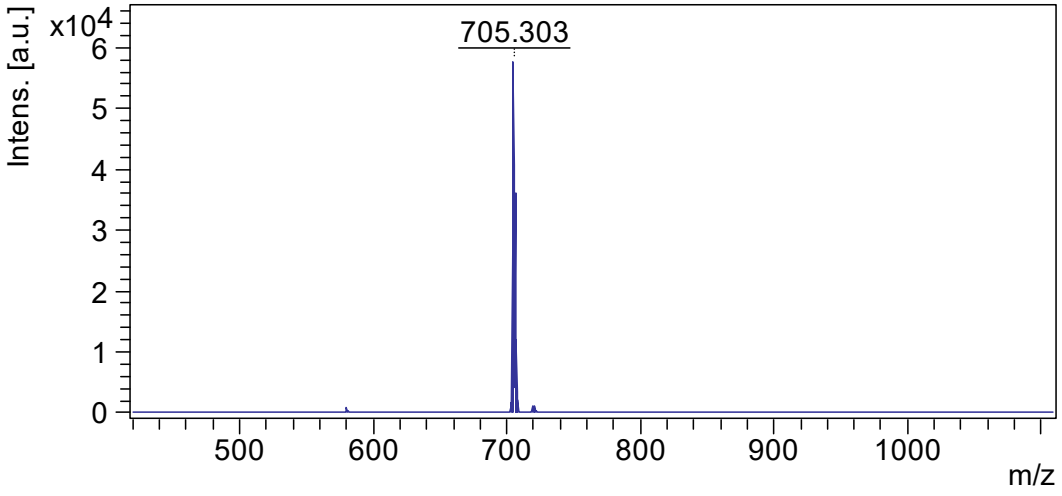


Fig. S3. MALDI-TOF of N-3QL.

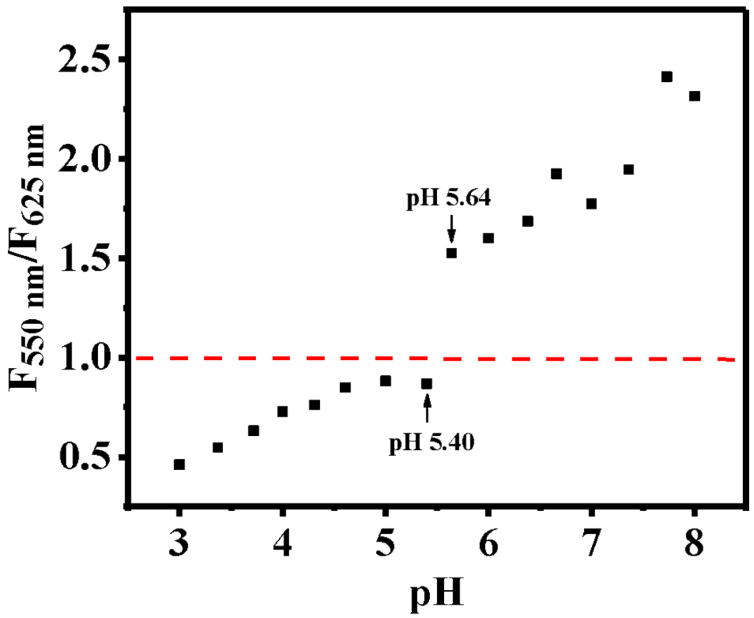


Fig. S4. Fluorescence ratio of N-3QL at different pH values.

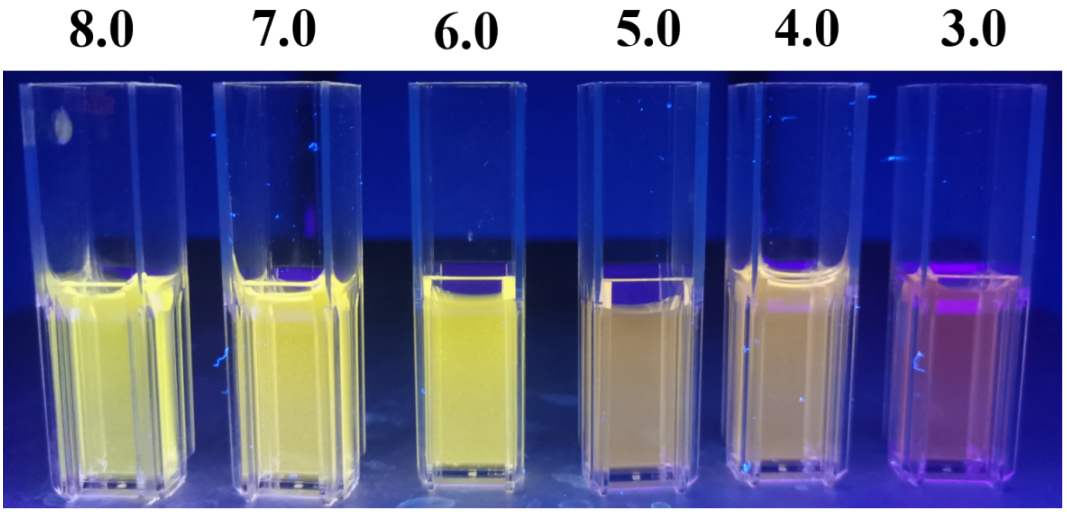


Fig. S5. Fluorescence diagram of N-3QL at different pH values.

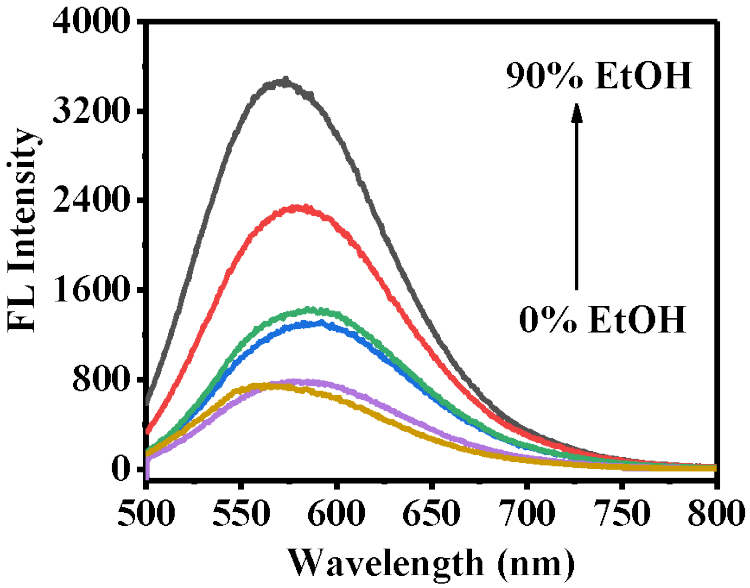


Fig. S6. Fluorescence emission spectra of N-3QL (10 μM) in DMF/EtOH mixture solution with different EtOH fractions (vol %).

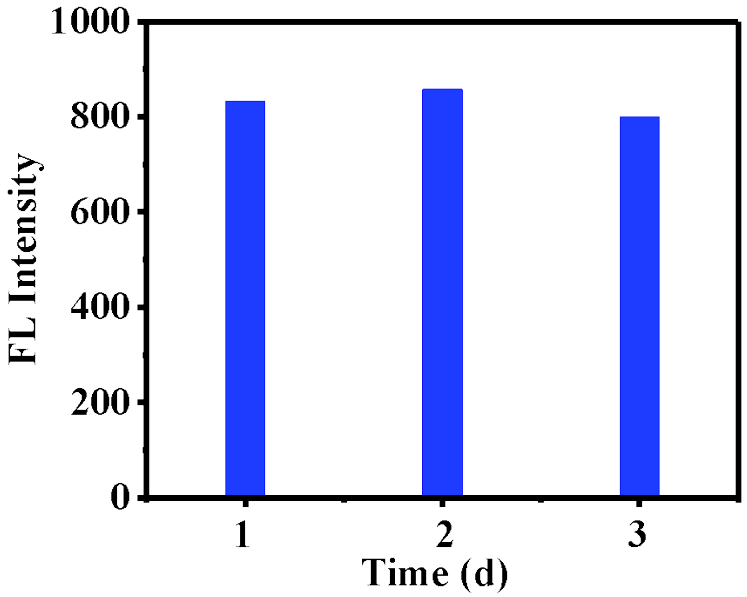


Fig. S7. Stability of N-3QL in cell culture media containing serum.

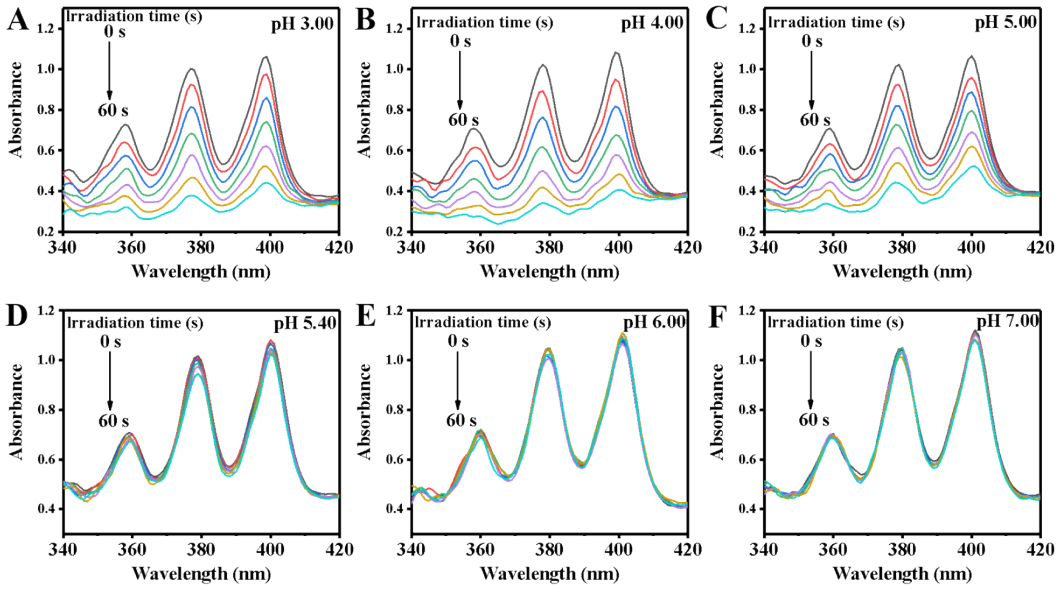


Fig. S8. UV-vis spectra of ABDA in buffer solutions containing N-3QL at different pH.

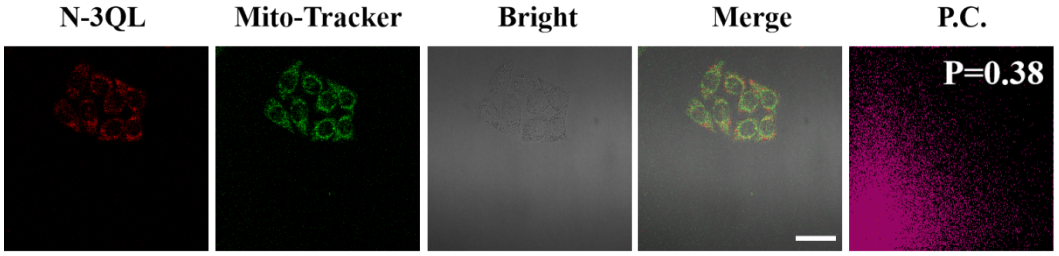


Fig. S9. Colocalization images of N-3QL in living HeLa cells (Mito Tracker Green: λ_{ex} = 488 nm, λ_{em} = 500-550 nm; N-3QL: λ_{ex} = 405 nm, λ_{em} = 625-675 nm). Scale bar: 20 μm.

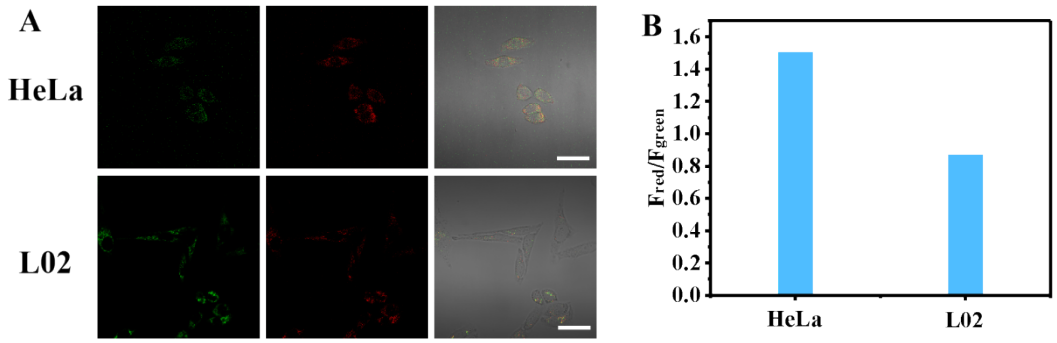


Fig. S10. (A) Fluorescence images of different cells with N-3QL (λ_{ex} = 405 nm, λ_{em} = 500-550 nm, λ_{em} = 625-675 nm). Scale bar: 20 μm. (B) The ratio of red band fluorescence to green band fluorescence in different cells.

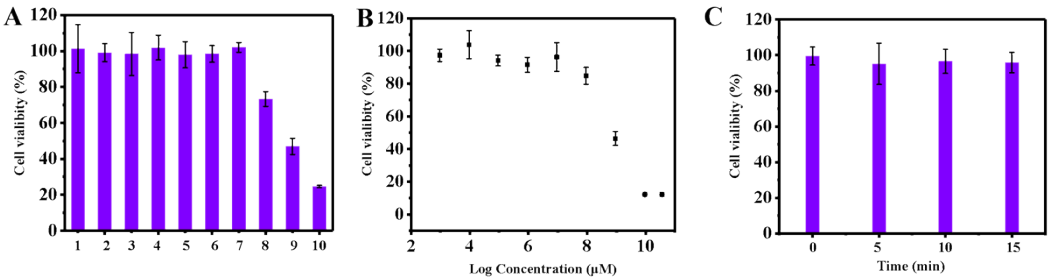


Fig. S11. (A) Cell viability of L02 cells treated with N-3QL and light irradiation (Concentration of N-3QL for "1-10": 0, 3.125, 6.25, 12.5, 25, 50, 75, 100, 150, and 200 μM). (B) Cell viability of HeLa cells treated with N-3QL but without light irradiation. (C) Cell viability of HeLa cells treated with different irradiation time (5 mW·cm^{-2} of 405 nm).

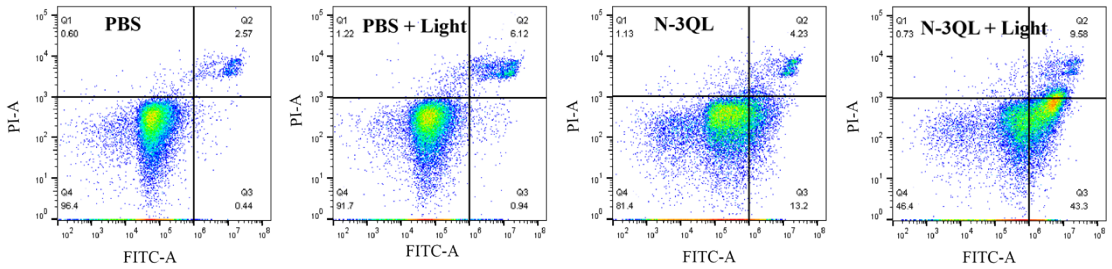


Fig. S12. Flow cytometry assays of HeLa cells stained with Annexin V-FITC and PI. (white light, 50 mW·cm^{-2}, 3 min).

REFERENCES

[1]. Li Y, Guo F, Zha Z, Wang Z. Chem. - Asian J. 2013, **8**, 534-537.