Supporting information

Real-time noninvasiveness monitoring cell mortality using a nucleic acid 'turn-on' quaternary ammonium probe with two-photon absorption

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Content

Methods and detailed experimental technique

Materials and apparatus

All chemicals and solvents were dried and purified by usual methods. DNA employed was Calf thymus DNA (ct-DNA) and RNA employed was ribonucleic acid diethylaminoethanol salt Type IX from Sigma-Aldrich for the in vitro experiments. IR spectra (4000–400 cm^{-1}), as KBr pellets, were recorded on a Nicolet FT–IR 170 SX spectrophotometer. Mass spectra were obtained on a Micromass GCT-MS Spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 400 spectrometer. UV-*vis* absorption spectra were performed by the UV-265 spectrophotometer. Fluorescence measurements were recorded on a Hitachi F-7000 fluorescence spectrophotometer. Before fluorescence experiments were performed, the fluorescence spectra were corrected by standard method. When the fluorescence measurements were taken, the concentration of samples was 1×10^{-5} M with quartz cuvette (path length = 1 cm). In the measurements of emission spectra, the slit width was 5 nm. The exciting voltage of emission spectrum was 400 V. For time-resolved fluorescence measurements, the fluorescence signals were collimated and focused onto the entrance slit of a monochromator with the output plane equipped with a photomultiplier tube (HORIBA HuoroMax-4P). The decays were analyzed by 'least-squares'. The quality of the exponential fits was evaluated by the goodness of fit (χ^2).

Spectroscopic measurements

The fluorescence quantum yields (Φ) were measured by using quinine sulfate as the reference, and the equation was shown as below:

$$\Phi_s = \Phi_r \frac{\int F_s}{\int F_r} \frac{A_r}{A_s} \frac{n_s^2}{n_r^2}$$

Where the *s* and *r* indexed designate the sample and reference samples,

respectively, A was the absorbance at λ_{exc}. n was the average refractive index of the appropriate solution. F was the integrated area under the corrected emission spectrum. Φ was the quantum yield.

Crystal analysis and theory calculation

Single-crystal X-ray diffraction measurements were carried out on a Bruker Smart 1000 CCD diffractometer equipped with a graphite crystal monochromator situated in the incident beam for data collection at room temperature. The determination of unit cell parameters and data collections were performed with Mo-K_α radiation (λ = 0.71073 Å). Unit cell dimensions were obtained with least-squares refinements, and all structures were solved by direct methods using SHELXL-97 program package. All non-hydrogen atoms were refined anisotropically and the hydrogen atoms were added theoretically and riding on the concerned atoms. The final refinement was performed by full-matrix least-squares methods with anisotropic thermal parameters for non-hydrogen atoms on F2.The calculations were carried out with the Gaussion 09 software package. The optimizations of the complex structures were performed using B3LYP density functional theory. On the basis of ground- and excited- state optimization, the TDDFT approach was applied to investigate the excited state electronic properties.

Molecular docking with DNA and RNA

Construct the strand DNA and RNA model, and the DNA single strand is 5' ATCGATCGAT. In the same way, the construction of RNA duplex was specified using the nucleotide sequence of 5' AUGCAUGCAU. The ligand of **QN2** is docked into the duplex DNA and duplex RNA using the method of CDOCK, respectively.

Cell culture

Cells were cultured in 25cm^2 culture flasks in DMEM, supplemented with fetal bovine serum (10 %), penicillin (100 units/mL) and streptomycin (50 units/mL) at 37 ^oC in a CO_2 incubator (95 %relative humidity, 5 % CO_2).Cells were seeded in 35 mm

glass bottom cell culture dishes, at a density of 1×10^5 cells and were allowed to grow when the cells reached more than 60 % confluence. The two compounds were dissolved in DMSO with concentration of 1 mM as stock solution, and the commercial dyes were prepared as 1 mM PBS solution and diluted to working concentration as protocol required.

MTT assay

The cytotoxicity of the **QN1**, **QN2** toward adherent cells was studied by MTT assay. HeLa cells were detached with trypsin, and seeded into 96-well plates (100 μL/well) at density of 5×10^4 cells/m land incubated in a humidified incubator at 37 ^{o}C for 24 h. Then adherent HeLa cells were treated with increasing concentrations of the compound (2.5×10^{-7} - 1×10^{-4} M) in the growth medium at 37 ℃ in 96-well plates. After 24 h incubation, MTT (5 mg/ml, 10 μl) was added to each well and incubated for additional 4 h. The supernatant was then removed and 100 μl of DMSO was added to dissolve the formazan crystals. And the cell culture plate was shaken for10 min until no particulate matter was visible. Absorbance in each well was measured at 570 nm using a microplate reader (Biotek, USA).The cell viability (%) was calculated according to the following equation: cell viability % = A/B ×100, where A represents the optical density of the wells treated with various concentration of the compounds and B represents that of the wells treated with medium.

Induced cell apoptosis and fixed

HeLa cells were chosen to use in confocal microscopy imaging. The cells were plated in 15 mm glass-bottom dish (NEST) and cultured for 48 h. For living cell staining experiments, cultured cells were stained with 1 μM **QN1**, **QN2** in culture medium for 20 min at 37 °C and then imaged with fluorescence microscopy. For fixed cell staining experiments, cultured cells were pretreated according to the following procedure: cells were first fixed by 4% paraformaldehyde for 15 min at room temperature and then with 1μM **QN2** in culture medium for 20 min at 37 °C. For the

investigating of the **QN2** staining effect, First, cells treated with 100 μM cisplatin, 100 μM 5-Fu and 20% DMSO for 4 h. Second, HeLa cells were induced **QN2** after triggered apoptosis with 10 μM 5-Fu for 12 h, 24 h, 48 h, then stained with AnnexinV-FITC/PI. Or cells treat with different content of DMSO for 5 h, then stain with **QN2.** Third, cell apoptosis was also induced by a commercially available apoptosis inducer A (1:2000, v/v) in culture medium for incubation of 12 h. confocal micrographs co-localized with PI. The excess complexes were washed away by PBS for 3 times, after that the confocal microscopy imaging was carried out. For other co-localization experimental, the triggered HeLa cells were incubated with DAPI, SYTO 9 for 10 min after washed way the excess tracker by PBS for 3 times.

DNase and RNase treatment

For DNase and RNase digest test, two sets of fixed HeLa cells were stained with 1 μM **QN2** in PBS (pH = 7.4) for 30 min. After washing with PBS twice, a total 1 mL PBS (as control experiment) was added into a set of cells and 25 mg/mL DNase-Free RNase or RNase-Free DNase was added into the other set of cells, and then two sets of cells were incubated at 37 °C in 5 % CO_2 for 2 h. In addition, the DNase and RNase digest tests of cells stained with 1 μM **QN2** was also carried out for comparison.

Fluorescence imaging

Confocal microscopy imaging was acquired with a Leica SP8 confocal microscopy and 100/63 × oil-immersion objective lens. The incubated cells were excited at 470 nm for compounds, 405 nm for DAPI, 488 nm for SYTO 9, and 550 nm for PI with a semiconductor laser, and the emission signals were collected at 600 ± 30 nm for compounds, 450 ± 30 nm for DAPI, 500 ± 15 nm for SYTO9, and 615 ± 20 nm for PI 7, respectively. Two-photon confocal microscopy imaging of the compounds was excited at 860 nm, while the emission signals were detected in the region of 550-650 nm. Quantization by line plots was accomplished by using the

software package provided by Carl Zeiss instrument.

Flow cytometry analysis

HeLa cells were seed in 6-well as described above. Cell apoptosis was also induced by a commercially available apoptosis inducer A (1:2000, v/v) in culture medium for incubation of 12 h. Cells were then stained with AnnexinV-FITC and propidium iodide (PI) according to the manufacturer's instructions. The another apoptosis triggered treat group stain with 1μM **QN2** in culture medium for 20 min at 37 °C, After that, the cells were trypsinized and washed with cold PBS twice. Then the samples were quantified by flow cytometry (Beckman Coulter, Inc.USA)

Animals: All procedures involving animals were approved by and conformed to the guidelines of the Anhui University Animal Care Committee, School of life science. We have taken great efforts to reduce the number of animal used in these studies and also taken effort to reduce animal suffering from pain and discomfort.

Tissue staining

Specific pathogen Free (SPF) KM (Kunming) mouse (male, two month, 18-22 g) was terminally anaesthetised and transcardially perfused with phosphate buffered saline (PBS) 0.1 M pH 7.4. The brain was extracted and then under usual fixative conditions. The frozen organs from PBS-perfused animals were sectioned at 20 μm and 200 μm in the sagittal plane using a cryostat (Leica 1950). tissue incubated with 1 μM **QN2** solution for 30 minutes at 37 ^{o}C in 95 % air 5 % CO_2, then washed with PBS buffer 3 times. Tissue was mounted cover-slipped using PI, and imaged directly using a Leica SP8 upright confocal system.

Synthetic routes

The synthesis of **QN1** and **QN2** presented in **Scheme 1**.The synthesis of **N1** presented in **Scheme 1** was according to the work previously.[1]

Prepared of **Q2.** (7.15 g, 50.0 mmol) 2-methylquinoline and (21.3 g, 150.0 mmol)

iodomethane were dissolved in 50 mL acetonitrile, and refluxed over night, then the yellow powder of **1** was got by vacuum filtering. (5.24 g, 18.4 mmol) **1** and (3.04 g, 46.0 mmol) and malononitrile were dissolved in 35 mL absolute ethanol, then 20 mL ethanol solution including (2.86 g, 42.17 mmol) sodium ethoxide was dropwise added into reactive solution in ice bath. After stirring 1 h under low temperature, the reactive solution continued to stir for 4 h under room temperature. When the reaction was finished, the reactive solution was poured into ice water, the pH was adjusted to 7.4 by diluted hydrochloric acid. After filtering, the product was dried in vacuum and 3.17 g dark yellow powder was collected. yield: 78 %.

Prepared of 3. The 20 mL chloroformic solution including (20.87 g, 72.7 mmol) phosphorus oxybromide was dropwise added into (3.54 g, 48.47 mmol) dried DMF in ice-salt baths until appearing frozen salt, then (4.39 g, 24.23 mmol) 2,2'-(phenylazanediyl)diethanol was added, the reactive solution was heated to 85°C and stirred overnight. When the reaction was finished, the reactive solution was poured into ice water, the pH was adjusted to 7.4 by 1 mol/L NaOH solution, then product was extracted by dichloromethane, washed three times, the product obtained after evaporation under reduced pressure and was purified by column chromatography (petroleum ether : ethyl acetate = 50 : 1, v/v). 3.87 g white powder was got, yield: 62 %.

Preparation of N2. **3** (1.00 g, 3.9 mmol) with 20 mL acetonitrile and 60 mL 40 % aqueous trimethylamine were sealed in a 50 mL teflon reactor autoclave and heated to 90 ^{o}C for 10 h. After cooling to room temperature, trimethylamine, acetonitrile and water were removed under vacuum. The crude product was washed three times using dichloromethane and dried in vacuum. 0.87 g brown powder was got, yield: 71 %.

Preparation of QN1. (1.06 g, 3.62 mmol) **N1** and (0.88 g, 3.62 mmol) **Q2** were dissolved into 15 mL acetonitrile, then 0.5 mL piperidine was added into the stirring mixed solution, the reactive solution was refluxed 12 h. When the reaction was finished, the mixture was filtrated under reduced pressure and was purified by column

chromatography (petroleum ether : ethyl acetate = 10 : 1, v/v). 0.52 g yellow powder was got, yield: 29 %. $C_{29}H_{28}N_4O_4$, Calcd (%): C, 70.15; H, 5.68; N, 11.28. Found: C, 70.12; H, 5.67; N, 11.25. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm) δ = 8.91 (d, *J* = 7.4Hz, 1H), 8.05 (d, *J* = 8.8Hz, 1H), 7.93 (d, *J* = 7.2Hz, 1H), 7.71 – 7.56 (m, 3H), 7.33 (s, 2H), 7.04 (s, 1H), 6.64 (d, *J* = 8.9Hz, 2H), 4.30 (s, 4H), 4.14 (q, *J* = 7.1Hz, 4H), 4.00 (s, 3H), 1.21 (t, *J* = 7.1Hz, 6H).^{13}C-NMR (d_6-DMSO, 100 MHz, ppm) δ = 170.04, 151.95, 150.50, 149.18, 139.69, 138.55, 133.15, 129.57, 124.75, 123.54, 120.50, 118.08, 115.91, 111.99, 105.96, 60.52, 52.56, 37.34, 14.09.FT-IR (KBr, ν, cm^{-1}): 2983(w), 2190(s), 2166(m), 1740(s), 1700(s), 1685(w), 1606(m), 1545(s), 1523(s), 1499(w), 1457(m), 1434(s), 1419(m), 1371(w), 1349(w), 1330(m), 1281(m), 1190(s), 1166(m), 1026(m), 968(m), 866(w), 834(w), 816(m), 771(m), 758(m). HRMS-ESI: *m/z*, *cal*: 497.22, *found*: 497.22 [M$^+$]. M.p. = 227-230 °C。

Preparation of QN2. (1.00 g, 2.20 mmol) **N2** and (0.49 g, 2.20 mmol) **Q2** were dissolved into 15 mL acetonitrile, then 0.5 mL piperidine was added into the stirring mixed solution, the reactive solution was refluxed for 12 h. When the reaction was cooled to room temperature, the mixture was filtrated under reduced pressure, the dark red powder was got. Then the dark red powder was dissolved into distilled water, the aqueous solution including (0.72 g, 4.40 mmol) NH_4PF_6 was added into the stirring solution, and continue to stir 2h at room temperature. The mixture was filtrated under reduced pressure, washed three times by diethyl ether and dried in vacuum. 0.52 g red powder was got, yield: 31 %. $C_{31}H_{40}F_{12}N_6P_2$, Calcd (%): C, 47.33; H, 5.13; N, 10.68. Found: C, 47.30; H, 5.14; N, 10.71. ^{1}H-NMR (d_6-DMSO, 400 MHz, ppm) δ = 8.92 (d, *J* = 8.2Hz, 1H), 8.05 (d, *J* = 8.8Hz, 1H), 7.94 (d, *J* = 8.0Hz, 1H), 7.76 (d, *J* = 8.7Hz, 2H), 7.63 (d, *J* = 7.8Hz, 1H), 7.40 (s, 2H), 7.03 (s, 1H), 6.91 (d, *J* = 8.7Hz, 2H), 3.99 (s, 3H), 3.90 (d, 4H), 3.49 (d, 4H), 3.18 (s, 18H). ^{13}C-NMR (d_6-DMSO, 100 MHz, ppm) δ = 152.55, 150.41, 147.26, 139.38, 139.21, 133.36, 129.99, 125.05, 129.93, 124.79, 120.47, 118.59, 117.22, 112.88, 105.97, 60.55, 52.69, 46.08, 43.28, 37.37.FT-IR (KBr, ν, cm^{-1}): 3041(w), 2196(s), 2173(m), 1605(m), 1587(s),

1549(s), 1523(s), 1490(m), 1481(m), 1442(m), 1416(w), 1354(m), 1336(m), 1293(w), 1278(s), 1236(w), 1192(s), 1174(w), 1155(w), 1121(w), 1078(w), 968(m), 920(m), 840(s), 772(w), 741(w), 559(s). HRMS-ESI: *m/z*, *cal*: 248.17, *found*: 248.16 [M^+]. M.p. = 327-330 °C。

Table S1. Crystal data collection and structure refinement of **QN1**

Compound	**QN1**
Empirical formula	$C_{29}H_{28}N_4O_4$
Formula weight	496.55
CCDC	1575888
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system, Space group	Triclinic, Pī
a /Å	8.358(2) Å
b /Å	9.923(2) Å
c /Å	7.348(4) Å
α	105.023(3) °
β	93.779(3) °
γ	109.005(3) °

Volume	1296.0(5) °
Z, Calculated density	2, 1.272 g/cm^{-3}
Absorption coefficient	0.086 mm^{-1}
F(000)	524
Crystal size	0.190 × 0.180 × 0.170 mm^3
Theta range for data collection	4.482 to 49.996 °
Limiting indices	$-9 \le h \le 9$, $-11 \le k \le 11$, $-20 \le l \le 20$
Reflections collected	9278/23101
R (int)	0.0184
Data/restraints/parameters	4517/0/337
Goodness-of-fit on F^2	0.971
Final R indices	$R_1 = 0.0499$ $wR_2 = 0.1255$

Table S2. Selected Bond Lengths (Å) and Bond Angles (°) of **QN1**

Selected bonds	Value(Å)	Selected angles	(°)
C6-N1	1.390(2)	C7-C9-C8	112.33(18)

C12-N1	1.359(3)	C27-O3-C28	117.6(3)
C13-N1	1.477(3)	C23-O4-C24	117.3(2)
C21-N4	1.381(3)	O1-C27-O3	124.6(3)
C23-O2	1.183(3)	O1-C27-C26	125.0(3)
C23-O4	1.320(3)	O3-C27-C26	110.4(2)
C14-C15	1.321(3)	C29-C28-O3	110.1(4)
C5-C4	1.365(3)	C25-C24-O4	110.5(3)
C8-N3	1.152(3)	O2-C23-O4	124.0(3)
C7-N2	1.146(3)	O2-C23-C22	125.6(2)
C22-N4	1.443(3)	O4-C23-C22	110.5(2)
C24-O4	1.455(4)	C21-N4-C22	122.2(2)
C26-N4	1.440(3)	C21-N4-C26	122.32(19)
C27-O1	1.180(3)	C22-N4-C26	115.25(19)
C27-O3	1.313(3)		
C28-O3	1.461(4)		

Table S3. Linear photophysical properties of **QN1** and **QN2** in different solvents.

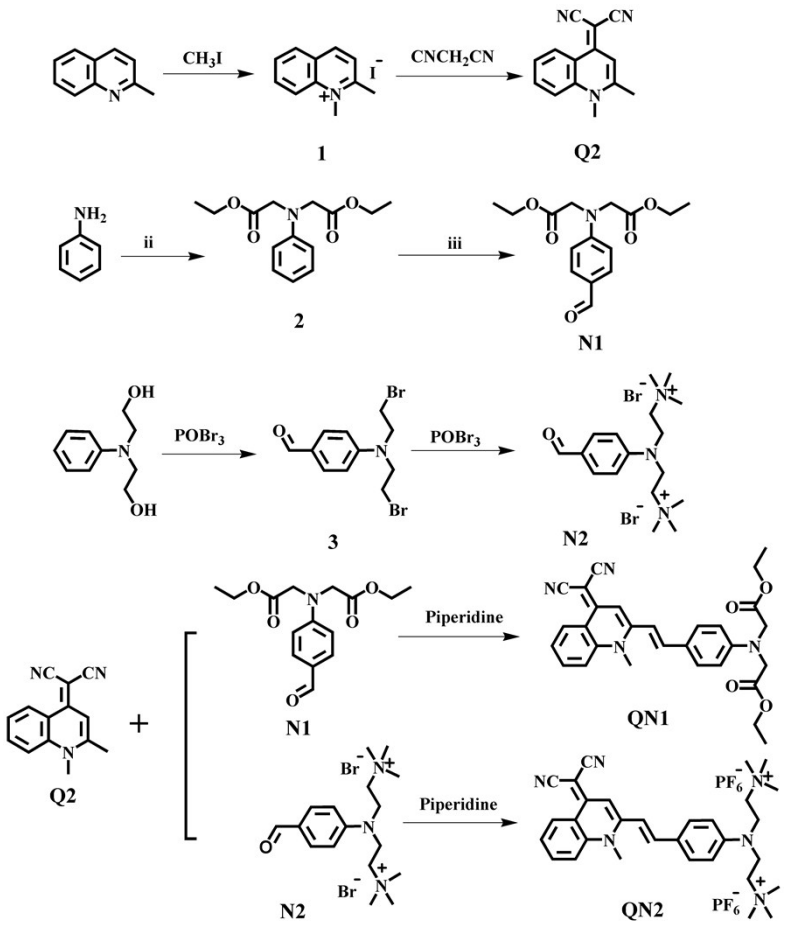
Compounds	solvents	λ_{max}^{abs}	ε	λ_{max}^{em}	Stocks shift
QN1	Benzene	399,460	2.63,2.47	535	136,75
	CH_2Cl_2	406,460	2.64,2.51	555	149,95
	THF	408,460	3.00,2.71	532	124,72
	EtOH	407,454	3.09,2.84	556	149,102

	MeCN	403,453	2.98,2.75	535	132,82
	DMSO	419,461	3.98,3.51	569	150,108
QN2	Benzene	415,459	1.51,1.43	575	160,116
	CH_2Cl_2	391,454	0.62,0.74	574	183,120
	THF	392,458	2.38,2.40	582	190,124
	EtOH	390,450	2.38,2.53	569	179,119
	MeCN	385,450	2.29,2.30	555	170,105
	DMSO	412,459	3.19,3.21	559	147,100

λ_{max}^{abs} is the peak position of the longest absorption band. ε is th extinction coefficient ($\times 10^4$ $mol^{-1} \cdot L \cdot cm^{-1}$). λ_{max}^{em} is the peak position of SPEF, excited at the absorption maximum.

Table S4. Calculated triplet transitions and the frontier orbitals of **QN1** and **QN2**.

	Energy /eV	Wavelength /nm	Oscillator	Molecular orbital	Main transition character
QN1	3.05	406	0.7059	131 (H) → 132 (L)	π→π*, ICT
	3.40	365	0.1586	130 (H-1) → 132 (L)	$\pi_{CN} \rightarrow \pi^*_{CH=CH}$
QN2	2.99	414	0.7512	109 (H) → 110 (L)	π→π*, ICT
	3.40	365	0.1800	108 (H-1) → 110 (L)	$\pi_{CN} \rightarrow \pi^*_{CH=CH}$



Scheme S1. Schematic representation of the synthesis procedures of **QN1** and **QN2**

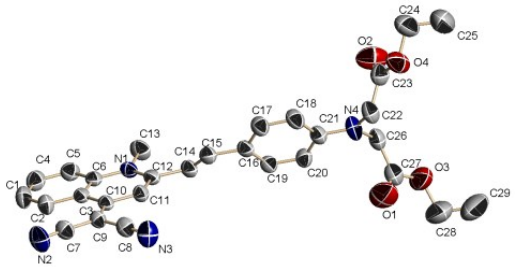


Figure S1. Side view of crystal structure of **QN1**.

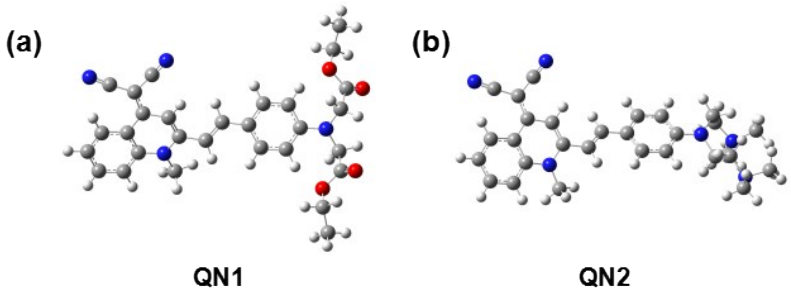


Figure S2. The optimized structures of **QN1** and **QN2**.

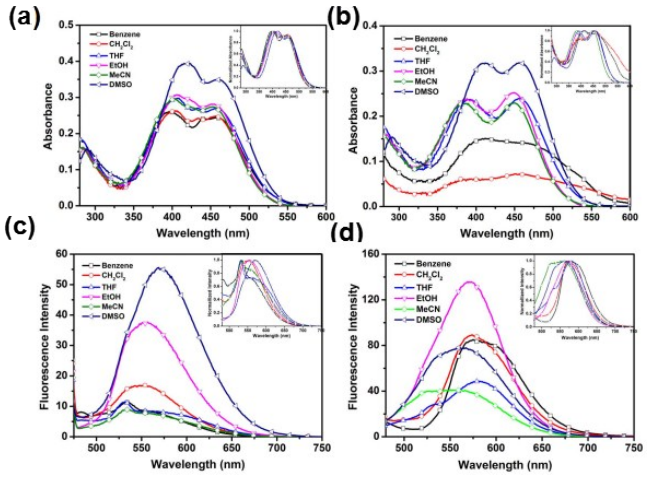


Figure S3. UV-vis absorption and Fluorescence emission spectra of **QN1** (a), (c) and **QN2** (b), (d) in different solvents.

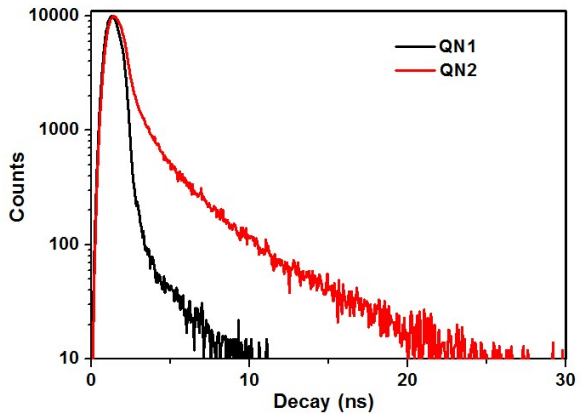


Figure S4. Fluorescence decay of **QN1** and **QN2**

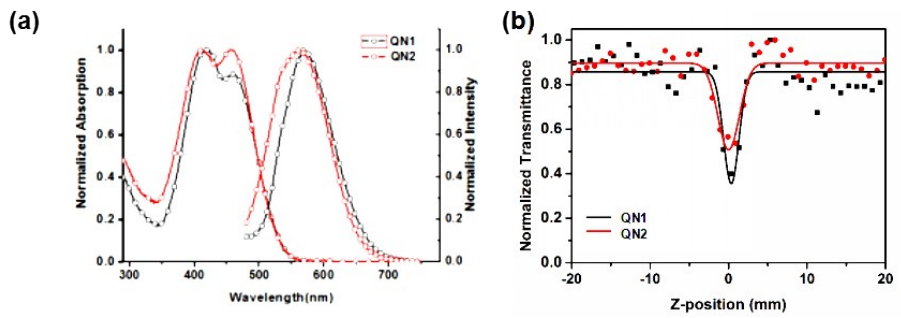


Figure S5. (a) UV-vis absorption and fluorescence emission spectra of **QN1** and **QN2** in 10 μM DMSO solution. (b) Open aperture Z-scanning of **QN1** and **QN2** in DMSO.

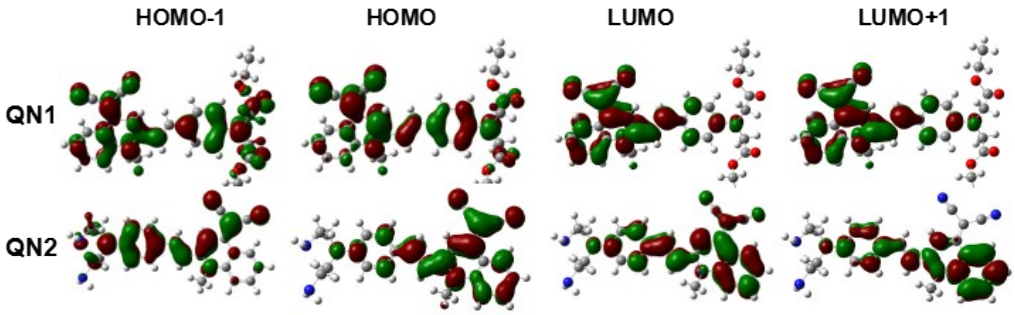


Figure S6. Representation of calculated HOMO and LUMO orbitals of **QN1** and **QN2**.

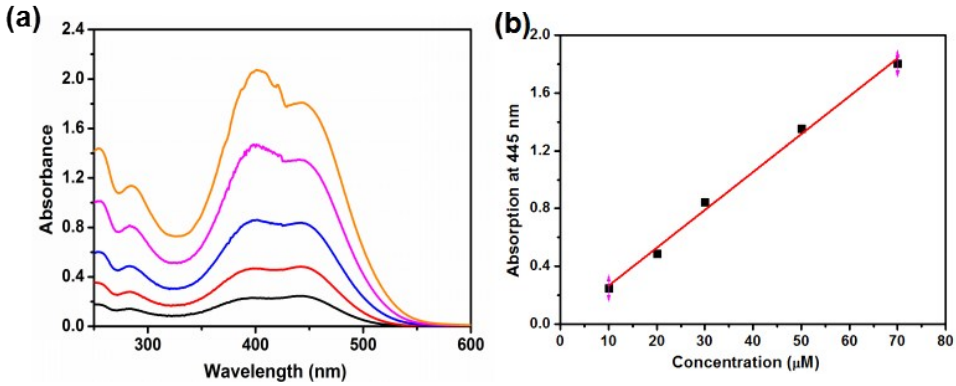


Figure S7. (a)Absorption spectra of **QN2** in H_2O buffered with HEPES. (b) Plot of intensity against the concentration.

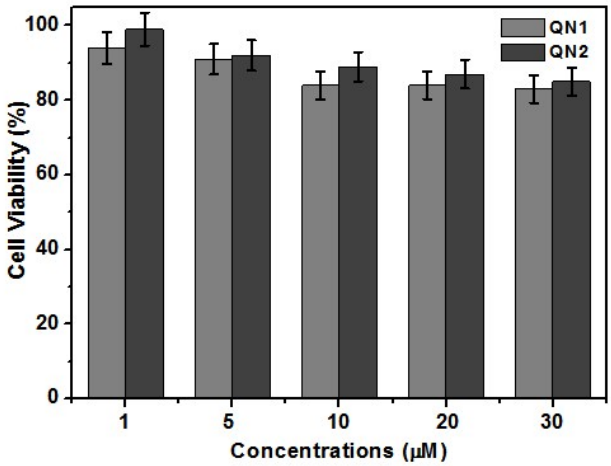


Figure S8. The MTT assay using HeLa for 24 hours treated with **QN1** and **QN2**.

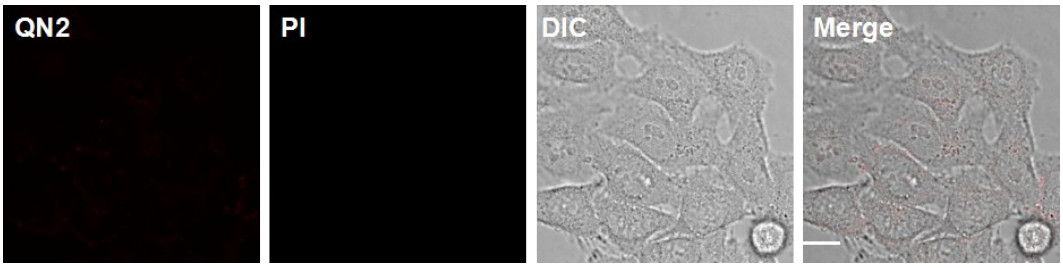


Figure S9. HeLa cells stained with and **QN2** for 24 h and then co-stained with PI. Scale bar is 20 μm.

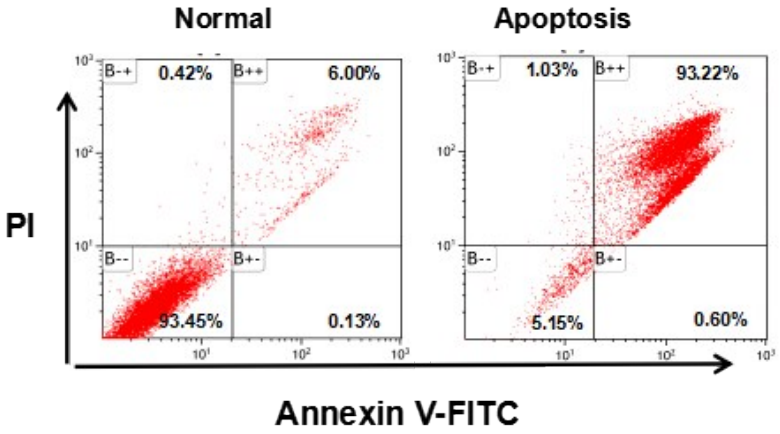


Figure S10. HeLa cells treated by apoptosis inducer for 12 h to trigger the cell apoptosis. Flow cytometry results of normal HeLa cells and apoptotic HeLa cells stained by AnnexinV-FITC and PI.

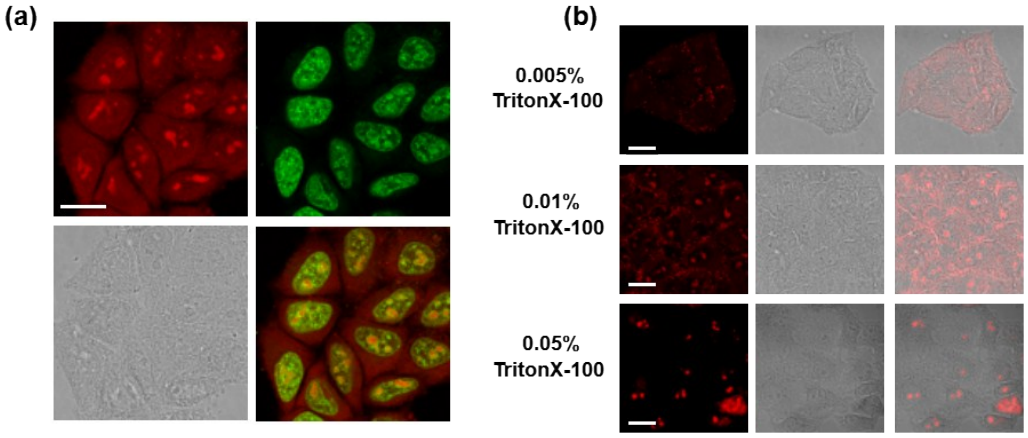


Figure S11. (a) HeLa cells after treatment with 0.01% TritonX-100 for 20min and co-localisation studies of **QN2** with PI .Scale bar is 25 μm. (b) Effects of cell damage agent on cellular uptake of **QN2** after induced with different concentration TritonX-100 for 20min, Scale bar is 20 μm.

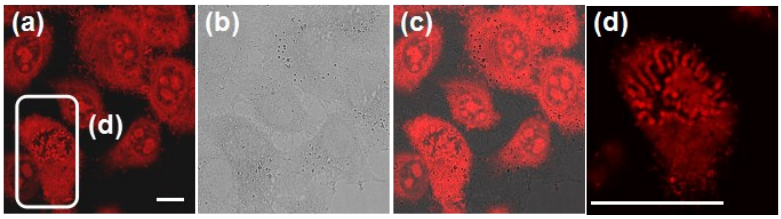


Figure S12. HeLa cells stained with **QN2** (5 μM, 15 min) after being fixed by paraformaldehyde (15 min). (a) Fluorescence images of **QN2**. (b) Bright field. (c) Merge image. (d) The enlarged image from the square marked in image (a).

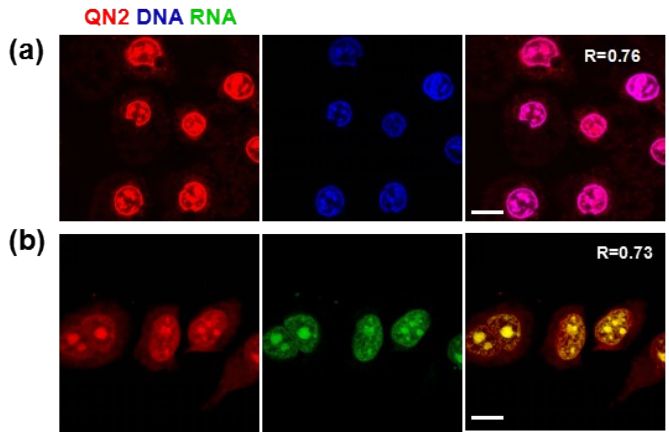


Figure S13. Fixed HeLa cells stain with **QN2** (5 μM,15 min) and co-localized with DAPI (a) and Syto 9 (b). Scale bar is 20 μm.

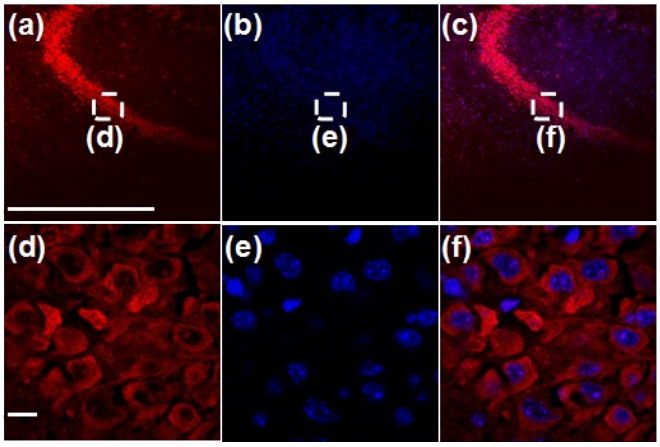


Figure S14. Mice brain slices stained with **QN2** (a), (d) and co-stained with DAPI (b), (e). The higher resolution micrographs showing the square marked in the bottom image. The scale bar represents 100 um.

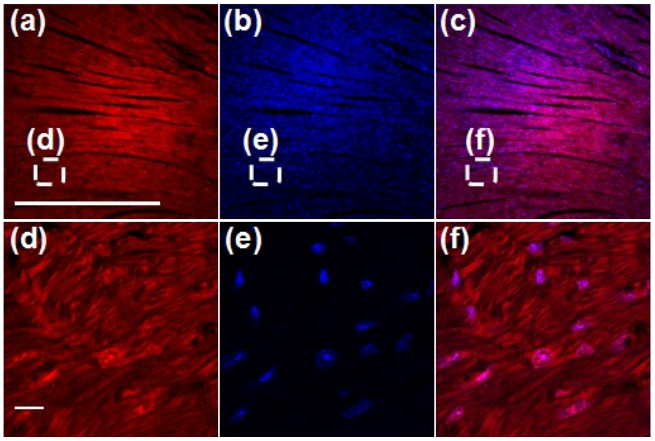


Figure S15. Mice heart slices stained with **QN2** (a), (d) and co-stained with DAPI (b), (e). The higher resolution micrographs showing the square marked in the bottom image. The scale bar represents 100 um.

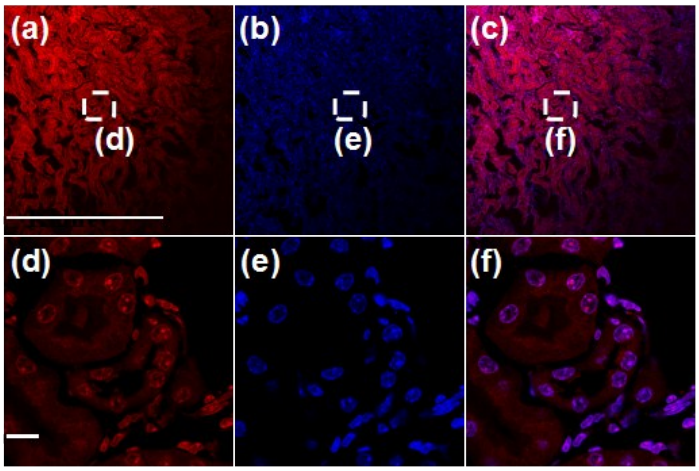


Figure S16. Mice kidney slices stained with **QN2** (a), (d) and co-stained with DAPI (b), (e). The higher resolution micrographs showing the square marked in the bottom image. The scale bar represents 100 um.

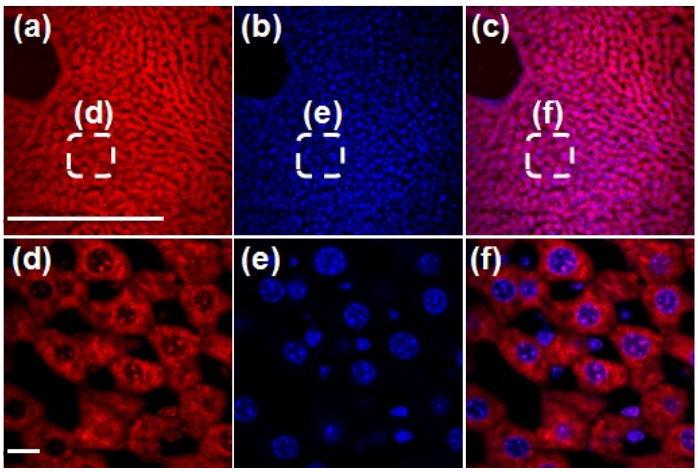


Figure S17. Mice liver slices stained with **QN2** (a), (d) and co-stained with DAPI (b), (e). The higher resolution micrographs showing the square marked in the bottom image. The scale bar represents 100 um.

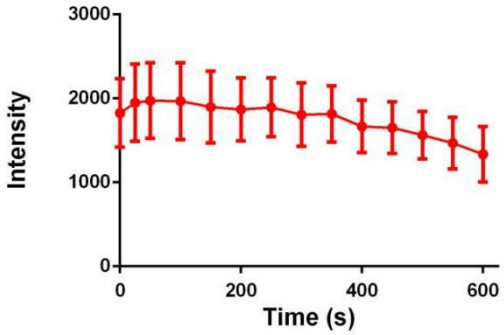


Figure S18. The fluorescence intensity decays for the cell images of **QN2**.

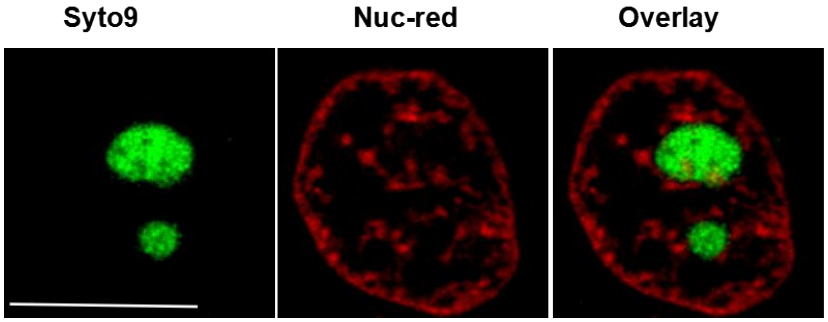


Figure S19. STED micrographs of HeLa cells stained with RNA-select dye Syto9/DNA-slect dye Nuc-red. The scale bar represents 10 um.

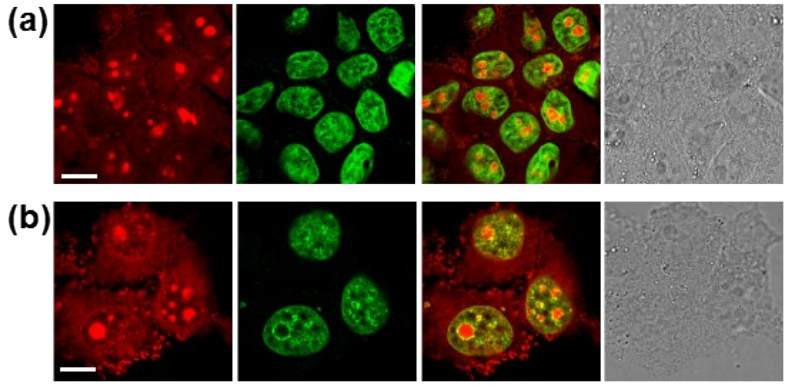


Figure S20. Effects of cell damage agent on cellular uptake of **QN2** in HeLa cells after treatment with 100 μM cisplatin (a), 100 μM 5-Fu (b) for 4 h. Confocal co-localisation studies of **QN2** with PI .Scale bar is 20 μm.

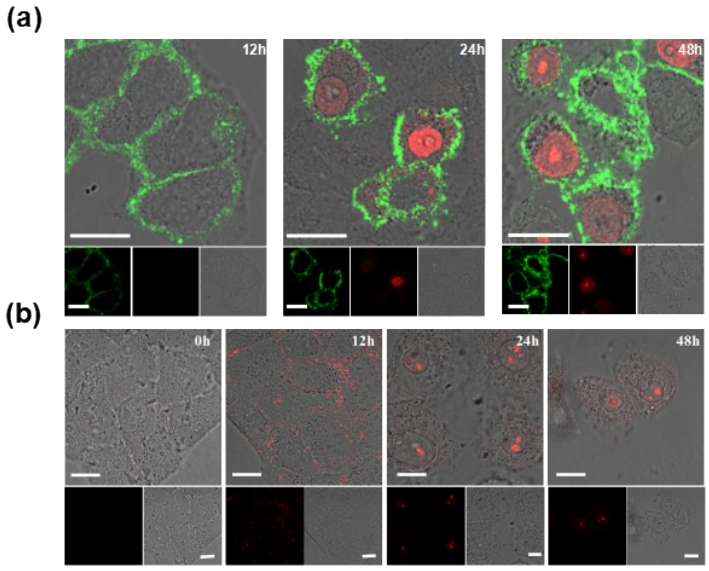


Figure S21. (a) HeLa cells were induced apoptosis with 10 μM 5-Fu for 12 h, 24 h, 48 h, then stained with AnnexinV-FITC/PI. (b) Two photon images of 5-Fu treated cell stain with **QN2**. Then incubuted with **QN2**.Scale bar is 20 μm.

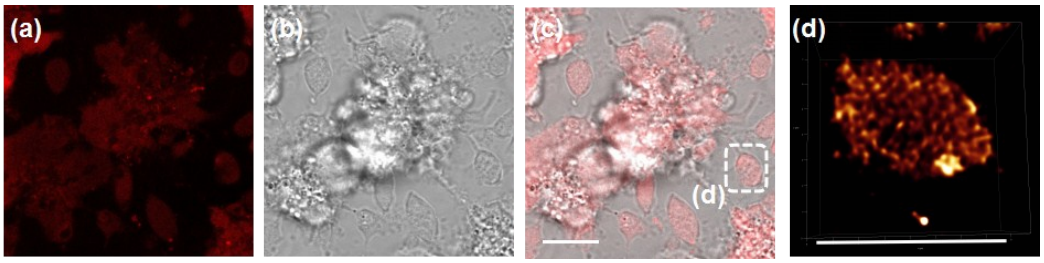


Figure S22. HeLa cells uptake **QN2** after treatment with 50 μM cisplatin for 12 h. (a) Fluorescence images of **QN2**. (b) Brightfield. (c) Merge image. (d) The 3D STED apoptotic body micrographs showing apoptosis-induced DNA fragmentation the marked in image (c). The scale bar represents 10 um.

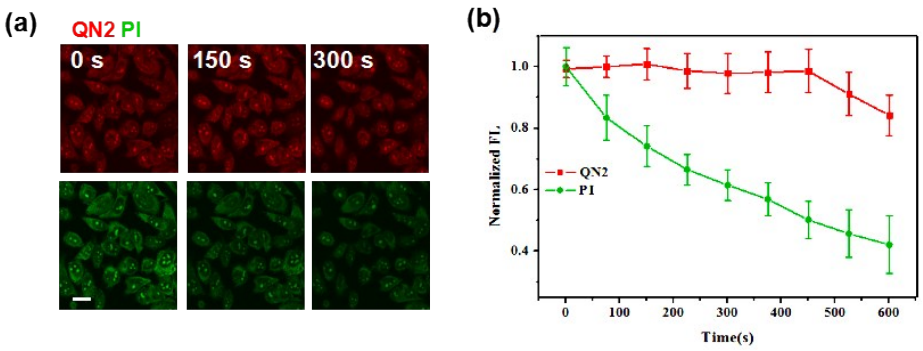


Figure S23. (a) The cell images of HeLa cells stain with **QN2** and PI after different irradiation times. (b) The fluorescence intensity decays for the cell images after different irradiation times. Scale bar is 10um.

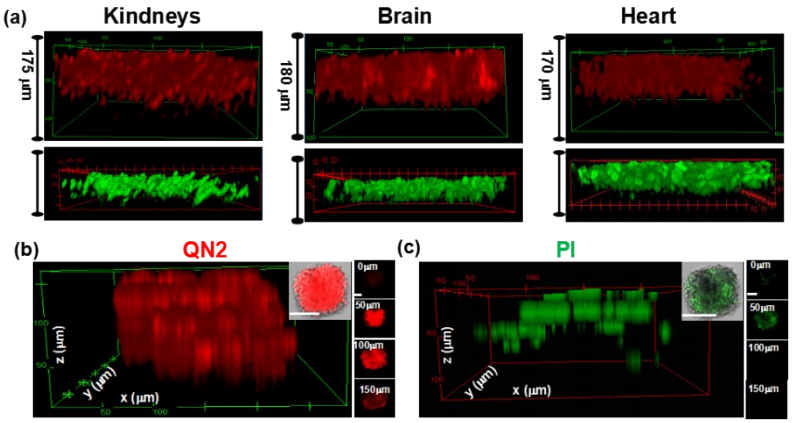


Figure S24. (a) The fluorescence intensity decays for the cell images in panel (right) after different irradiation times. Scale bar is 10um. Confocal micrographs of the 3D multicellular spheroids of SH-SY5Y cells, then stain with **QN2** (a) and PI (b) . 3D rendering of MCs showing detailed uptake of **QN2**, insert: the merge micrograhs of fluorescence and brighrfield images, respectively. Scale bar is 100um. (c). 3D Z-stack confocal image of the rat kindney, brain, heart slices stain with **QN2** and PI.

radio.avi

Video S1. The radio of real-time uptake of **QN2** in HeLa after apoptosis inducer trigger for 30 min.

Reference

[1] M. Zhao, Y. Zhu, J. Su, Q. Geng, X. Tian, J. Zhang, H. Zhou, S. Zhang, J. Wu and Y. Tian, *J. Mater. Chem. B*. **2016**, 4, 5907-5912.