

Supporting information

**Unique Polarity-Sensitive Photothermal Sensitizer Revealing Down-regulated Mitochondrial Polarity During Photo-Induced Cell Death**

Chuang Liu, Minggang Tian, Weiyong Lin\*

Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Materials Science and Engineering, University of Jinan, Jinan, Shandong 250022, P. R. China.

Email: [weiyonglin2013@163.com](mailto:weiyonglin2013@163.com)

# Contents

Materials .....	S3
Spectroscopic measurements .....	S3
Cell culture.....	S3
Figures and tables used in the manuscript.....	S4
Figure S1.....	S4
Table S1.....	S4
Table S2.....	S4
Table S3.....	S5
Table S4.....	S5
Figure S2.....	S5
Figure S3.....	S6
Figure S4.....	S6
Figure S5.....	S6
Figure S6.....	S7
Figure S7.....	S7
Figure S8.....	S8
Figure S9.....	S8

## **Materials**

All chemicals used are of analytical grade, 3-diethylaminophenol, 1,6-dihydroxynaphthalene, triphenylphosphine, 1,4-dibromobutane, etc. were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Sphingomyelin, 1, 2-dipalmitoyl-sn-glycero-3-phospho-(1'-rac-glycerol), cholesterol, lecithin, and 1, 2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). The solvents used in the spectral measurement are of chromatographic grade.

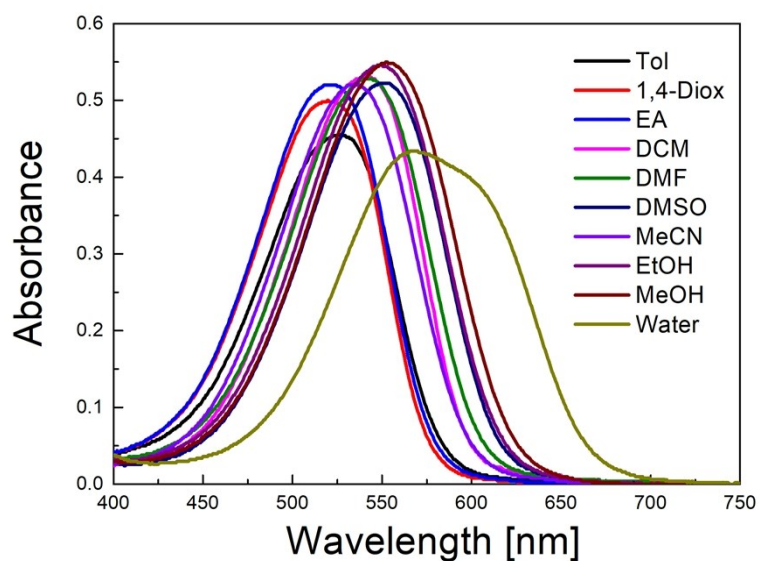
## **Spectroscopic measurements**

The UV-visible-near-IR absorption spectra of dilute solutions were recorded on a U2910 spectrophotometer using a quartz cuvette having 1 cm path length. One-photon fluorescence spectra of dilute solutions were obtained on a HITACH F-2700 spectrofluorimeter equipped with a 450-W Xe lamp. PBS buffer solution: 10 mM, NaCl, NaHPO<sub>4</sub>·12H<sub>2</sub>O, NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, pH = 7.40.

## **Cell culture**

Hepg2 cells were grown in H-DMEM (Dulbecco's Modified Eagle's Medium, High Glucose) supplemented with 10% FBS (Fetal Bovine Serum) in a 5% CO<sub>2</sub> incubator at 37 °C. For cell imaging experiments, live Hepg2 cells were suspended and diluted in the culture medium with cell concentration of 10000 cells/mL. 1 mL of the cell suspension solution was added into glass bottom dish and cultured for 24 h to allow adhesion.

## Figures and tables used in the manuscript



**Figure S1.** The absorption spectra of NRTP (10  $\mu$ M) in various solvents.

**Table S1.** The volume of stock solutions used to prepare GUVs

GUVs	$V_{[SM]}$ [ $\mu$ L]	$V_{[DPPG]}$ [ $\mu$ L]	$V_{[CL]}$ [ $\mu$ L]	$V_{[LE]}$ [ $\mu$ L]	$V_{[DOPG]}$ [ $\mu$ L]
SM	50	50	10	0	0
LE	0	0	0	50	50
LE + 10%CL	0	0	5	50	50
LE + 40%CL	0	0	20	50	50

**Table S2.** The emission wavelength and quantum yield of NRTP in various solvents.

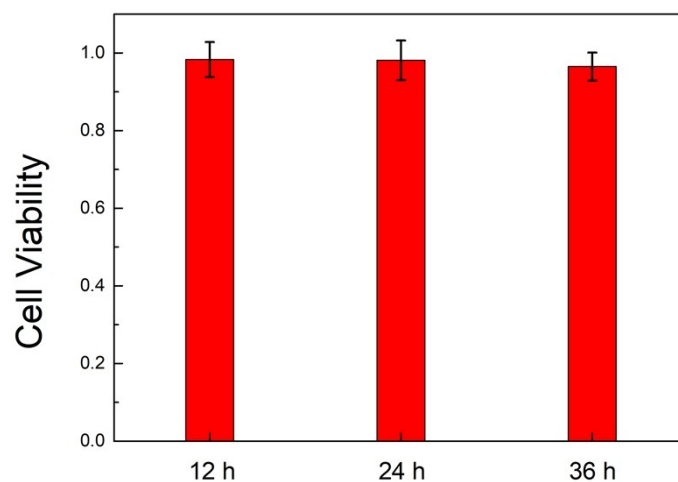
Solvent	Polarity	Emission [nm]	Quantum Yield
Toluene	33.9	605	0.242
1,4-Dioxane	36.0	608	0.256
EA	38.1	610	0.236
DCM	40.7	620	0.188
DMF	43.2	651	0.141
DMSO	45.1	647	0.096
MeCN	45.6	631	0.193
EtOH	51.9	634	0.064
MeOH	55.4	645	0.069
Water	63.1	672	0.002

**Table S3.** The emission wavelength and quantum yield of NRTP in mixed solvents of water and 1,4-dioxane.

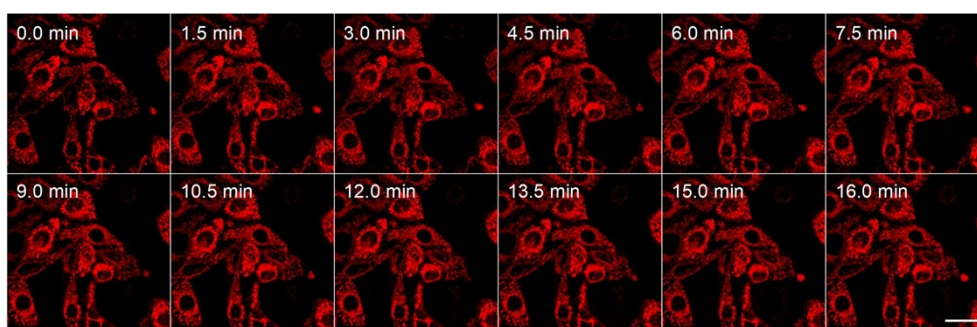
Water fraction	Emission [nm]	Quantum Yield
0%	608	0.256
1%	616	0.243
2%	620	0.212
4%	628	0.187
6%	632	0.159
8%	637	0.135
10%	639	0.125
15%	644	0.116
20%	646	0.091
25%	649	0.071
30%	651	0.052
35%	653	0.050
40%	655	0.037
60%	663	0.019
80%	669	0.007
100%	672	0.002

**Table S4.** The temperature of 5 mM NRTP in mixed solvent of DMSO and PBS buffer (v:v = 1:4) after irradiation by mercury lamp (200-800 nm) for different time.

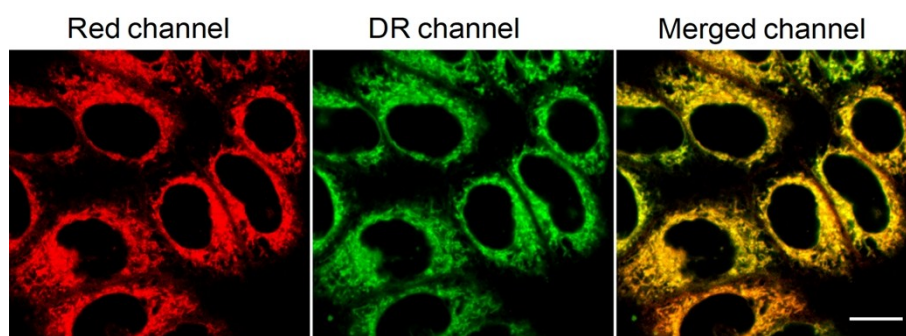
Irradiation time [min]	Temperature (°C)
0	20
5	40
10	45
15	48



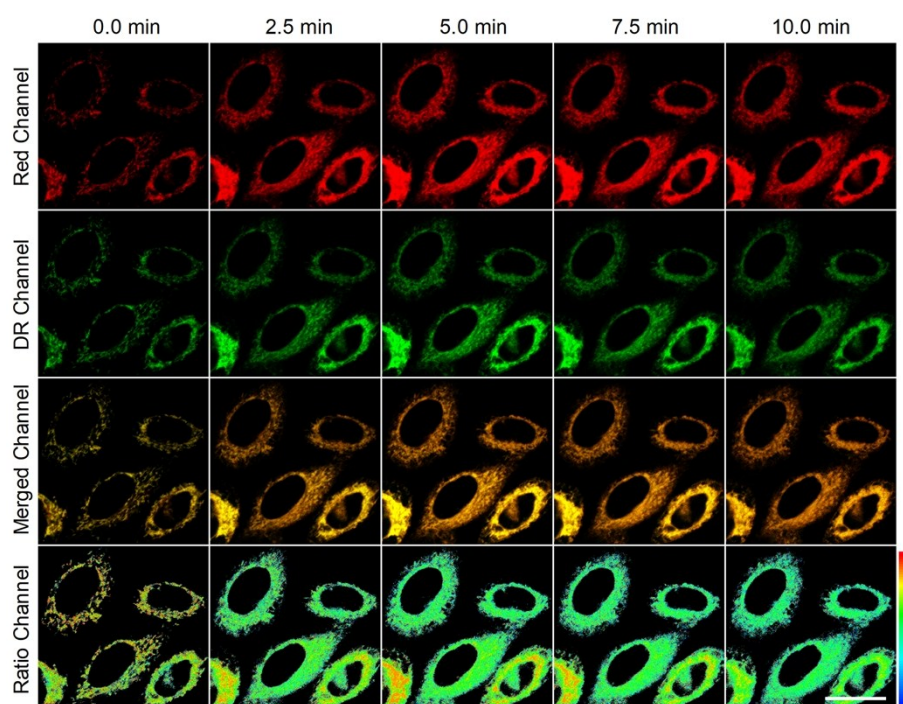
**Figure S2.** The cell viability of HepG2 cells incubated by 5  $\mu$ M NRTP for different time.



**Figure S3.** The fluorescence images of HepG2 cells incubated by 5  $\mu$ M NRTP under the ceaseless exposure to 561 nm laser for different time (0-16 min).



**Figure S4.** The fluorescent images of HepG2 cells co-stained by 5  $\mu$ M NRTP and 200 nM MTDR for 30 min, exposed by high-power 561 nm laser for 5 min, and then imaged under the excitation by low-power 561 nm laser (red channel, NRTP) and 647 nm laser (DR channel, MTDR). Bar = 20  $\mu$ m.



**Figure S5.** The fluorescent and ratiometric images of HepG2 cells incubated by 5  $\mu$ M NRTP then exposure to high-power 561 nm laser for different time (1-10 min).  $\lambda_{ex}$  = 561 nm. Bar = 20  $\mu$ m.

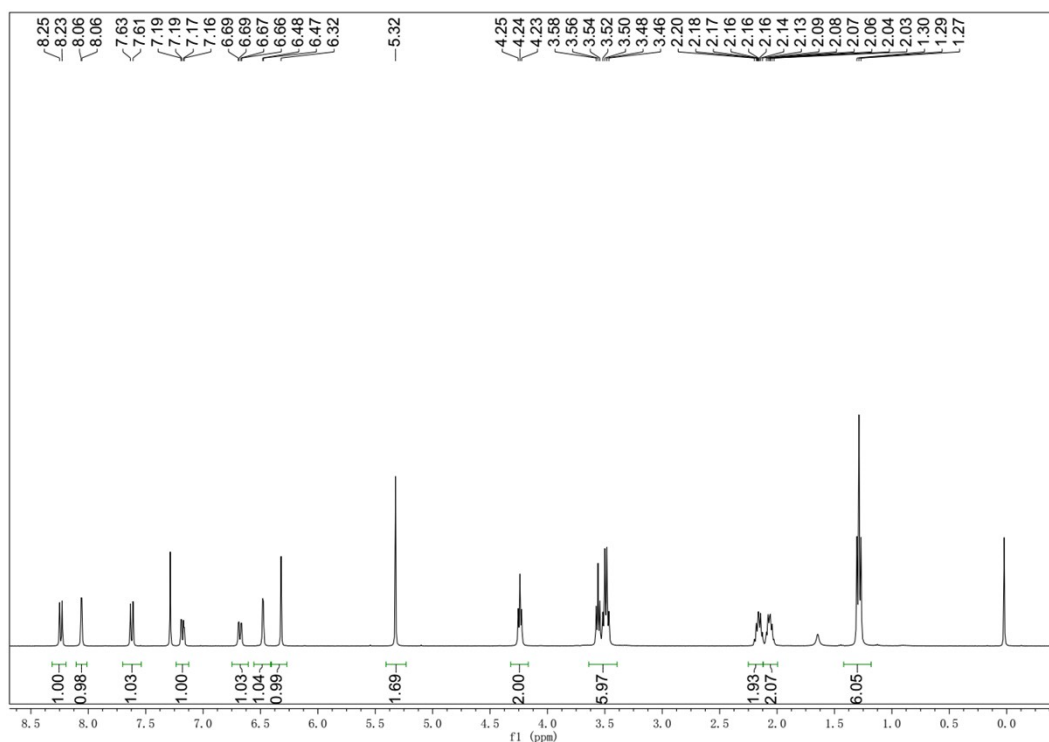


Figure S6. The  $^1\text{H}$  NMR spectra of **2**.

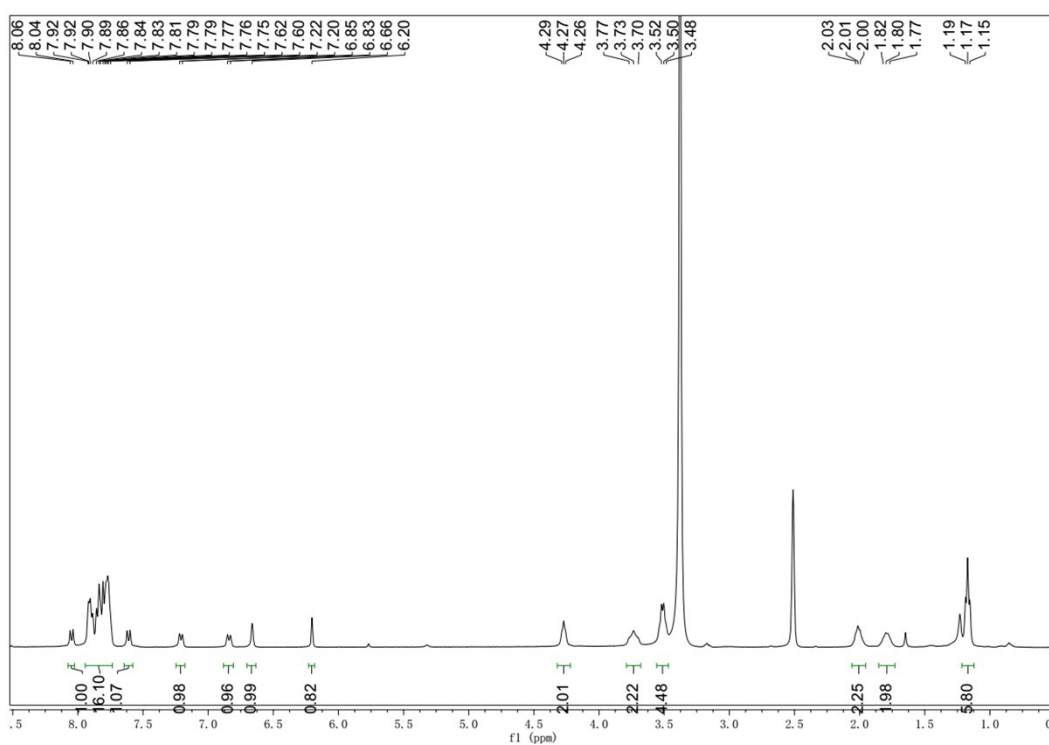
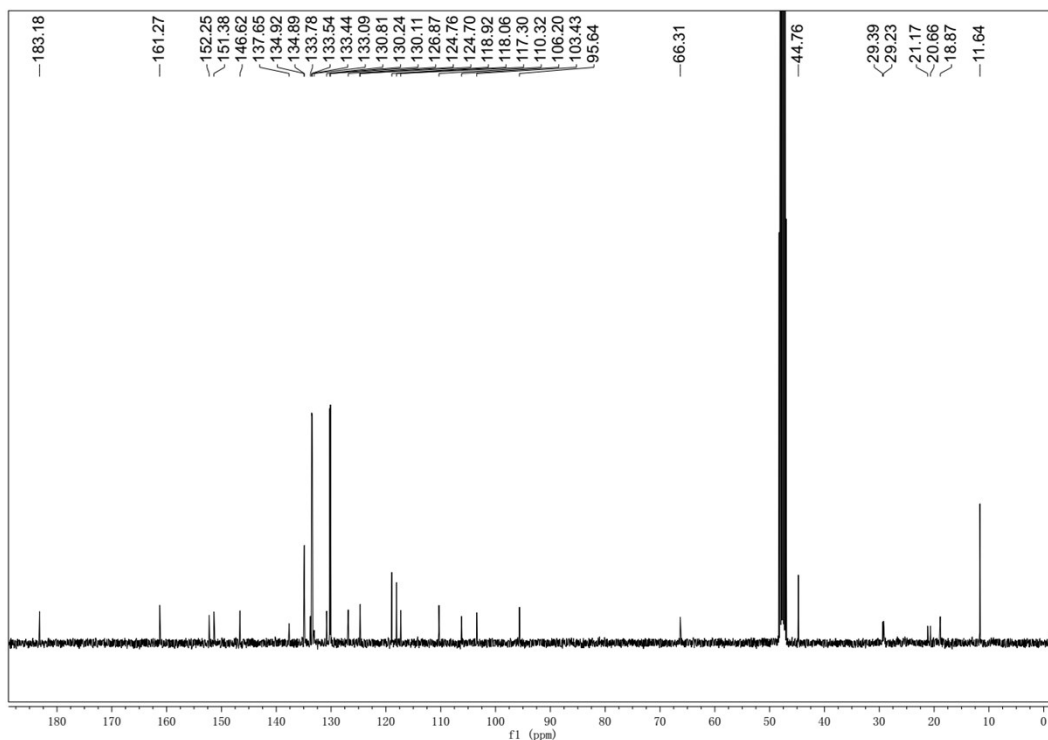
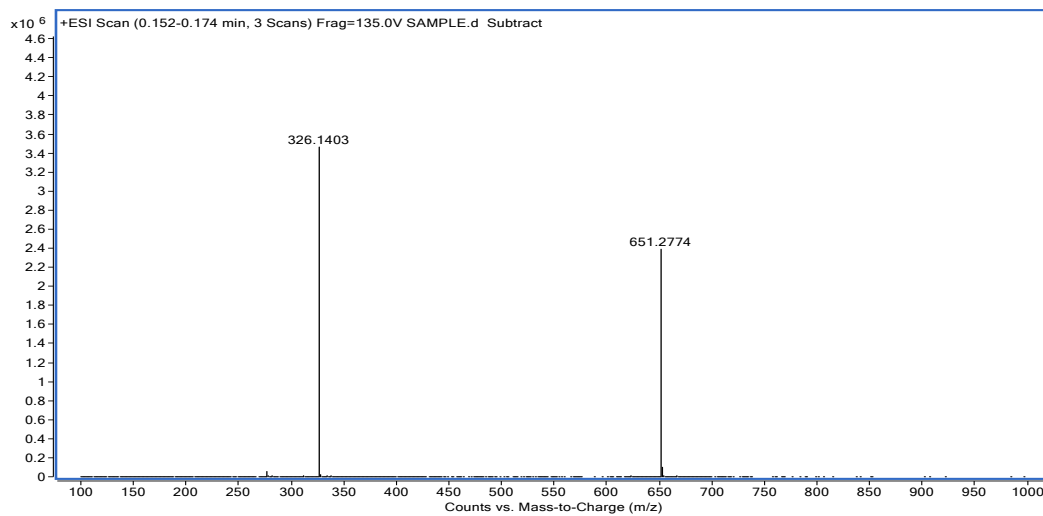


Figure S7. The  $^1\text{H}$  NMR spectra of NRTP.



**Figure S8.** The <sup>13</sup>C NMR spectra of NRTP.



**Figure S9.** The HRMS spectra of NRTP.