Supporting Information *for*

Hypoxia activated fluorescent probe for specific visualization of mitochondrial dysfunction in tumor

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Content

Materials

All chemicals used are of analytical grade, p-nitrobenzaldehyde, 4-methylquinoline, p-dimethylaminobenzaldehyde, et al. were purchased from Macklin Biochemical Technology Co., Ltd (Shanghai, China). Methyl iodide, pyrrolidine, etc. were purchased from J&K Chemical (Beijing, China). 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, $NaHPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, pH = 7.40.

Cell culture and imaging

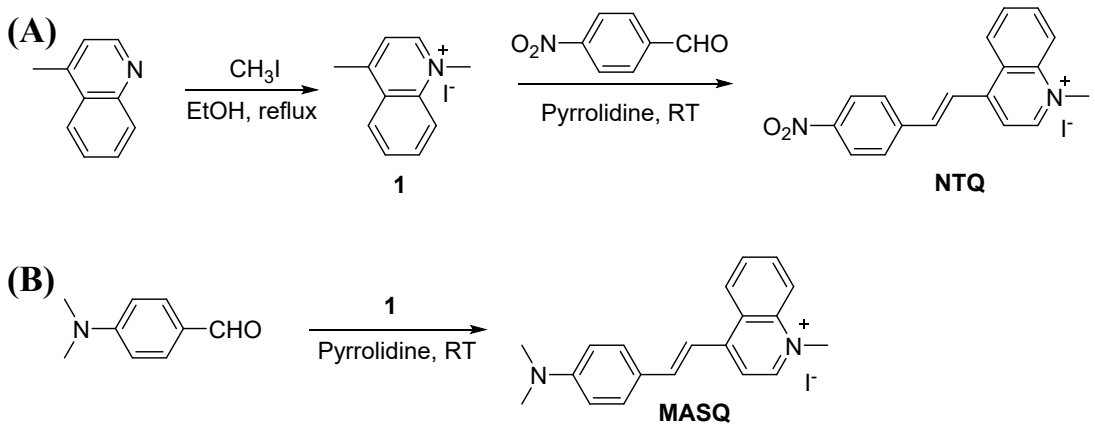
HeLa cells were purchased from Procell Life Science & Technology Co., Ltd., which were grown in H-DMEM (Dulbecco's Modified Eagle's Medium, High Glucose) supplemented with 10% FBS (Fetal Bovine Serum) in a 5% CO_2 incubator at 37 ˚C. For cell imaging experiments, live HeLa cells were suspended and diluted 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. For cell imaging experiments, the cells in dish were incubated with 10 μM **NTQ** and 1 μM **NTQ-Cont** for 30 min, which were directly imaged under confocal microscope without further washing procedure. For deep-red channel: λ_{ex} = 561 nm, λ_{em} = 665 - 735 nm;. **MTG**: λ_{ex} = 488 nm, λ_{em} = 500 - 550 nm.

Molecular simulation

Initially, the chemical structures of NTQ and ASQ were obtained with the Gaussian View software, which were optimized with semiempirical computational method (PM3 opt). The obtained structures were then optimized with DFT calculations in water (B3LYP/6-31G* scrf=(solvent=water, PCM) opt). The absorption wavelengths

and oscillator strengths (*f*) were obtained by performing time-dependent DFT calculations on the optimized ground-state structures (B3LYP/6-31G* scrf=(solvent=water, PCM) td=(nstate=6) freq). The excited states were subsequently optimized with time-dependent DFT calculations (B3LYP/6-31G* scrf=(solvent=water, PCM) td opt). Finally, the emission wavelengths and corresponding oscillator strengths (*f*) were obtained by performing time-dependent DFT calculations on the excited ground-state structures (B3LYP/6-31G* scrf=(solvent=water, PCM) td=(nstate=6) freq). These calculation methods were added into the supporting information.

Synthesis and characterizations



Scheme S1. The synthetic routes of the probes **NTQ** (A) and **MASQ** (B)**.**

Syntheses of 1,4-dimethylquinolin-1-ium iodide (**1**). Into the 3 mL of ethanol, 4-methylquinoline (1.0 g, 7.0 mmol) and iodomethane (2.0 g, 14.0 mmol) were added. The reaction was refluxed at 80 °C for 12 h. After completion, the reaction was cooled to room temperature. The reaction solution was filtered, washed with petroleum ether and drum dried to give a white product (2.1 g, 86 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.37 (d, J = 5.9 Hz, 1H), 8.54 (d, J = 8.4 Hz, 1H), 8.49 (d, J = 8.9 Hz, 1H), 8.27 (t, J = 7.7 Hz, 1H), 8.10 – 8.02 (m, 2H), 4.58 (s, 3H), 3.01 (s, 3H).

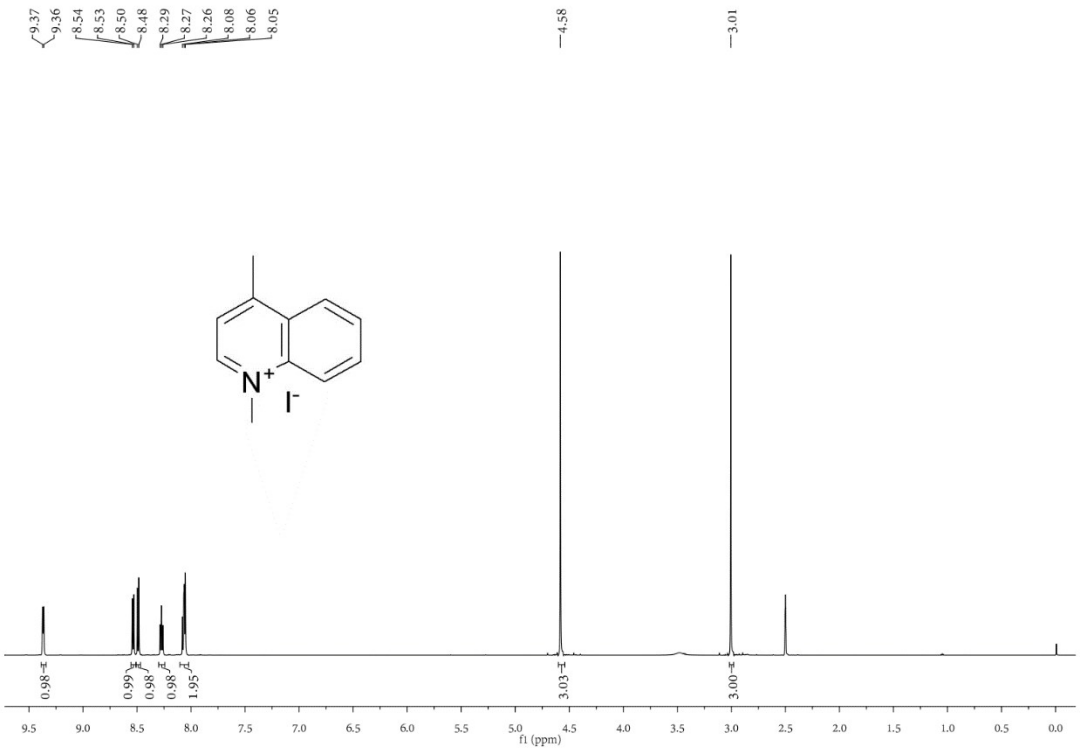


Figure S1. The ^{1}H NMR spectrum of compound **1** in DMSO-d_6.

Syntheses of 1-methyl-4-(4-nitrostyryl)quinolin-1-ium iodide (**NTQ**). 1,4-Dimethylquinoline-1-iodonium (941 mg, 3.3 mmol) and 4-nitrobenzaldehyde (500 mg, 3.3 mmol) were added to a reaction vial containing 4 mL of ethanol. Stir and add 200 μL of pyrrolidine. The reaction was carried out at room temperature for 6 h. A solid was precipitated. A reddish brown solid was obtained by filtration (1.0 g, 75 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.48 (d, J = 6.3 Hz, 1H), 9.07 (d, J = 8.5 Hz, 1H), 8.59 – 8.48 (m, 3H), 8.35 (d, J = 8.6 Hz, 2H), 8.32 – 8.28 (m, 1H), 8.25 (dd, J = 11.9, 10.2 Hz, 3H), 8.10 (t, J = 7.7 Hz, 1H), 4.60 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 151.06, 148.06, 147.29, 141.16, 139.18, 138.15, 134.59, 129.07, 126.00, 123.80, 123.49, 118.95, 116.76, 44.48. HRMS (ESI) m/z: $[M]^+$ Calcd for $C_{18}H_{15}N_2O_2^+$ 291.1128; Found 291.1137.

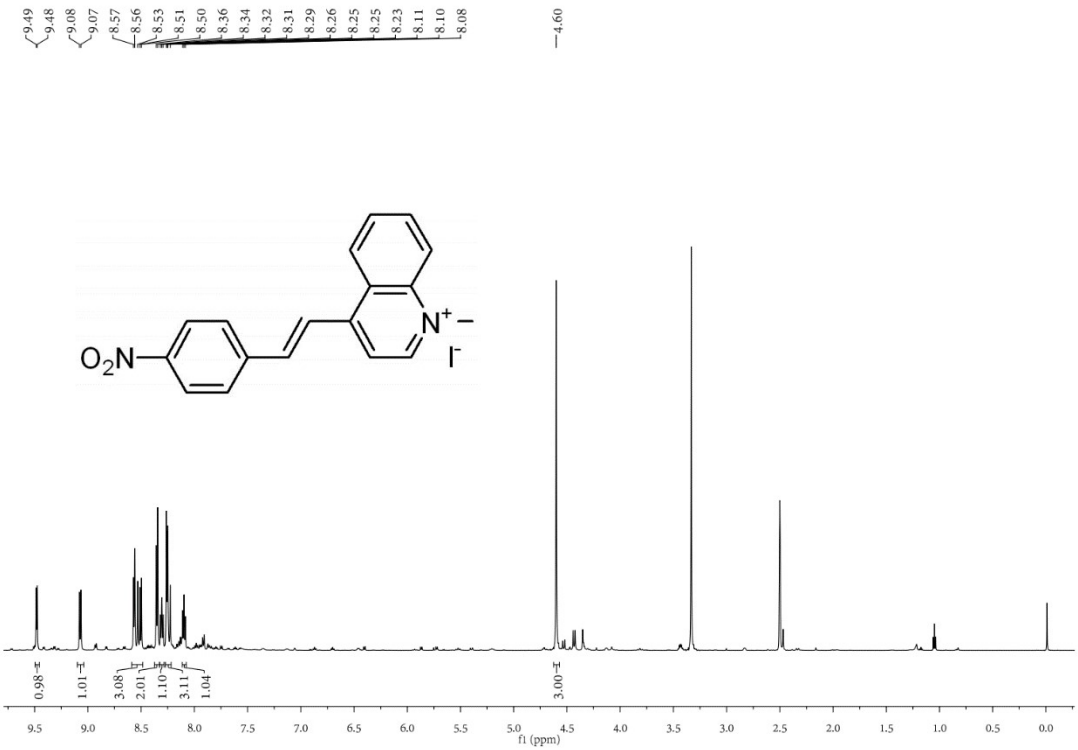


Figure S2. The ^{1}H NMR spectrum of **NTQ** in DMSO-d_6.

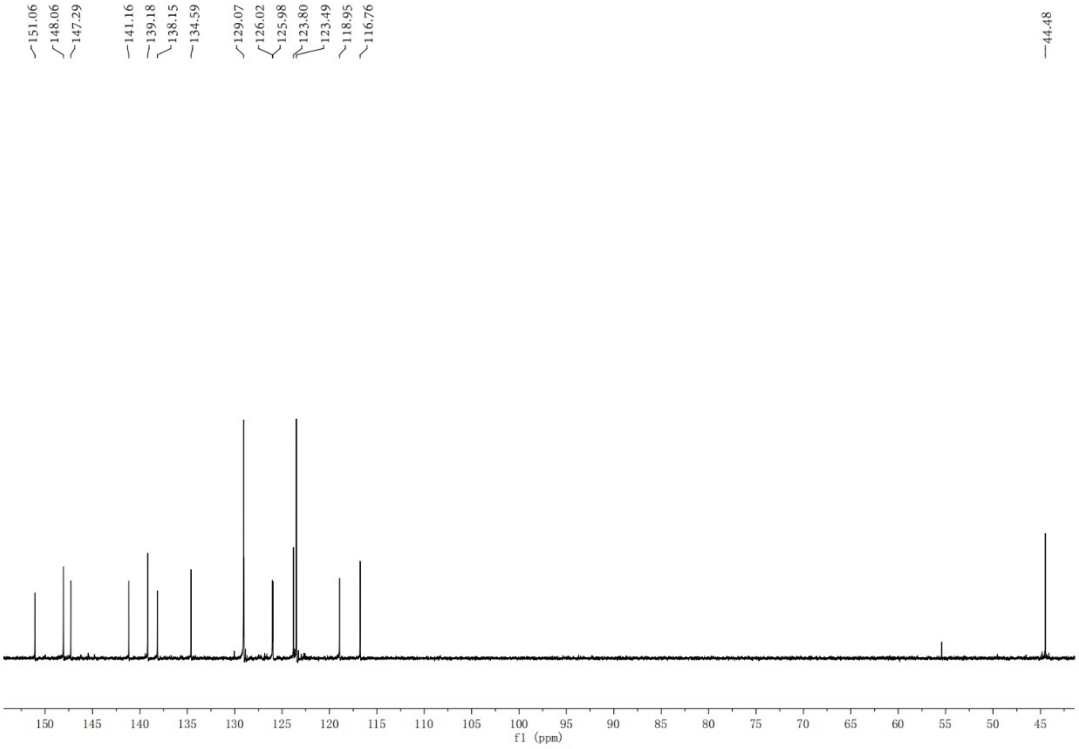


Figure S3. The ^{13}C NMR spectrum of **NTQ** in DMSO-d_6.

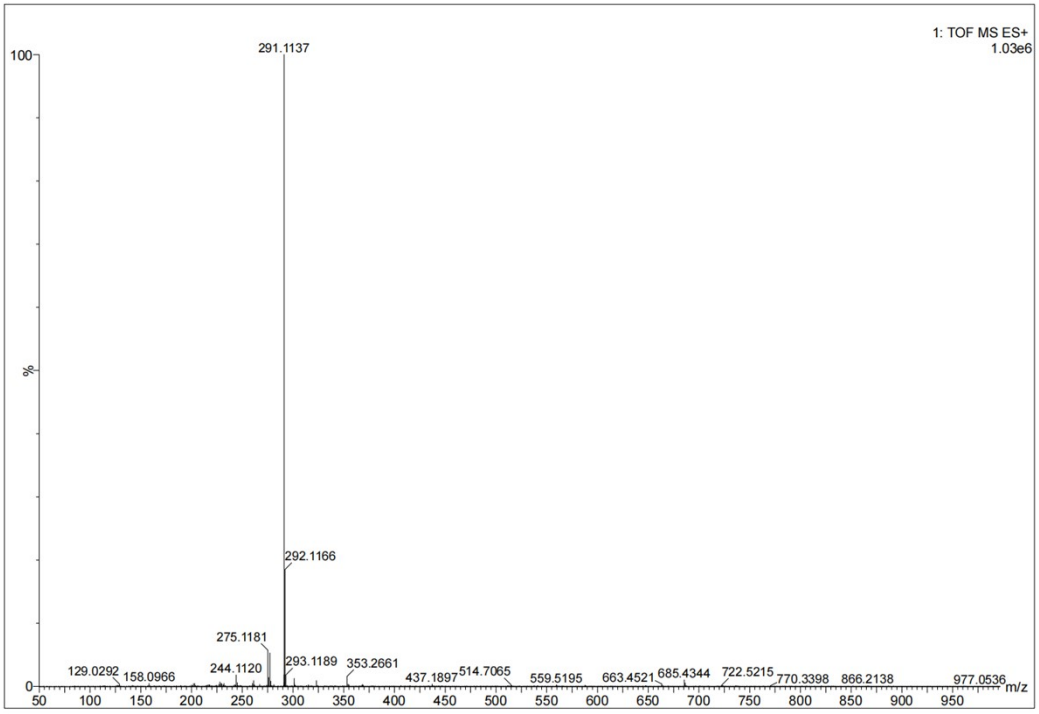


Figure S4. The HRMS spectrum of **NTQ**.

Syntheses of 4-(4-(dimethylamino)styryl)-1-methylquinolin-1-ium iodide (**MASQ**). **MASQ** was synthesized by a process similar to that of **NTQ**. The pure product was a black solid (963 mg, 69 %). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.11 (d, J = 6.7 Hz, 1H), 9.03 (d, J = 8.5 Hz, 1H), 8.37 – 8.29 (m, 2H), 8.19 (dd, J = 20.5, 11.7 Hz, 2H), 8.04 – 7.94 (m, 2H), 7.87 (d, J = 8.8 Hz, 2H), 6.82 (d, J = 8.8 Hz, 2H), 4.44 (s, 3H), 3.07 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 153.64, 152.78, 147.26, 145.17, 139.27, 135.09, 131.82, 129.03, 126.83, 126.23, 123.53, 119.53, 114.42, 113.59, 112.38, 44.49. HRMS (ESI) m/z: $[M]^+$ Calcd for $C_{20}H_{21}N_2^+$ 289.1699; Found 289.1707.

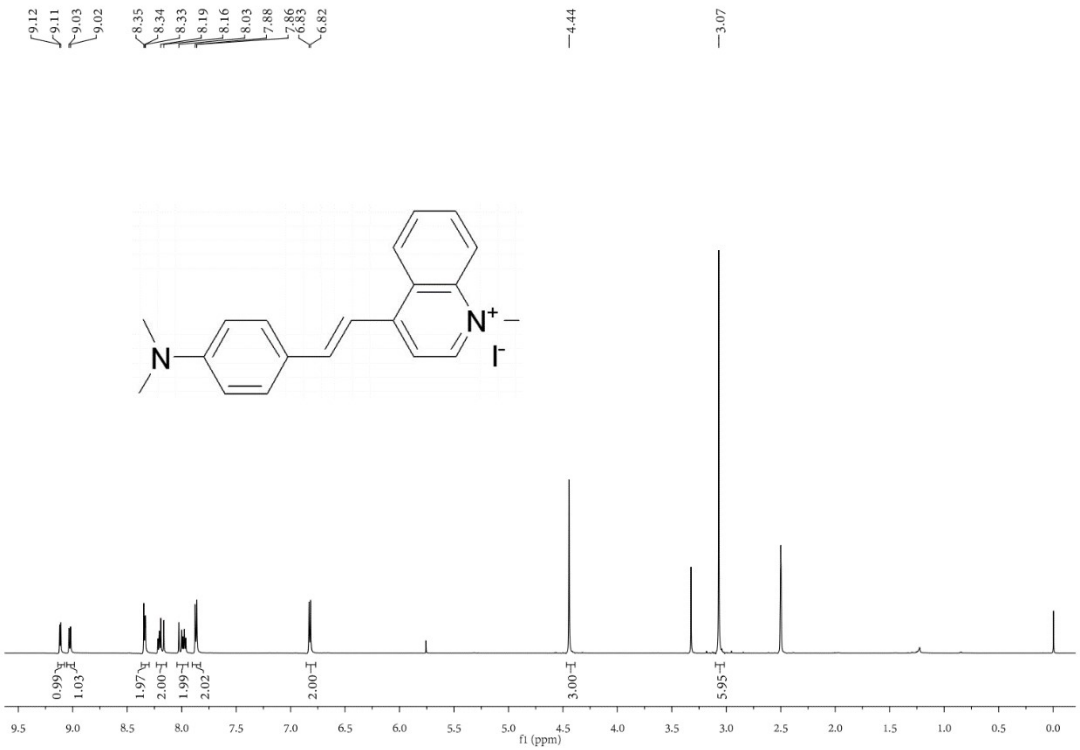


Figure S5. The ^{1}H NMR spectrum of **MASQ** in DMSO-d_6.

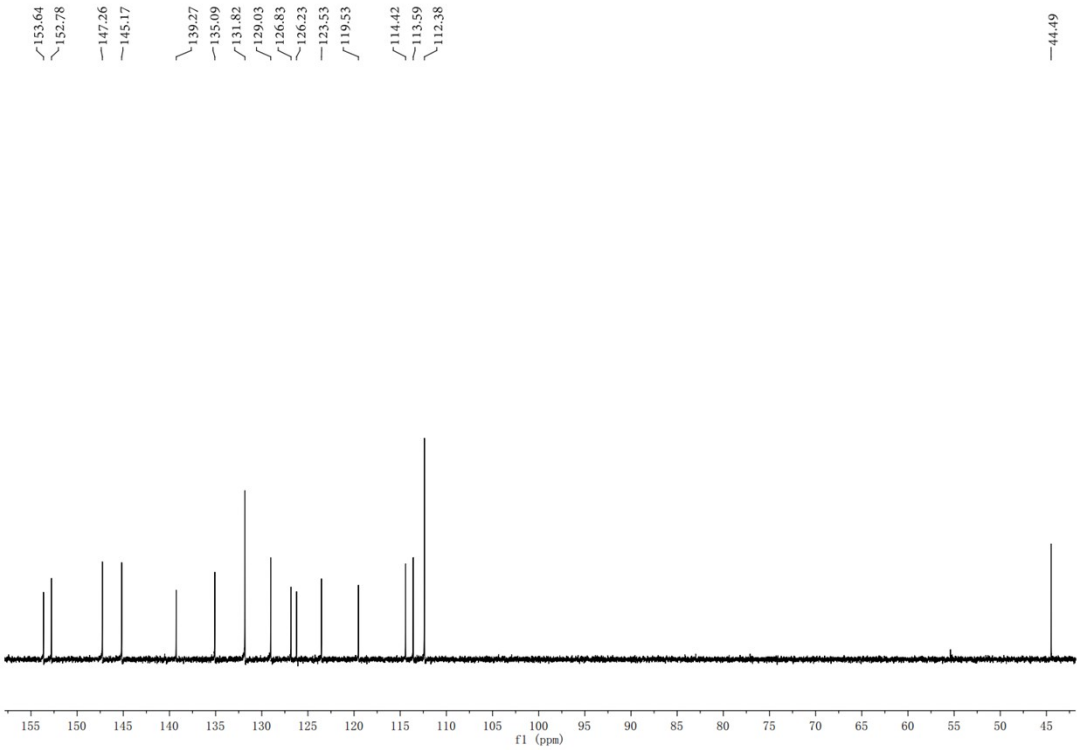


Figure S6. The ^{13}C NMR spectrum of **MASQ** in DMSO-d_6.

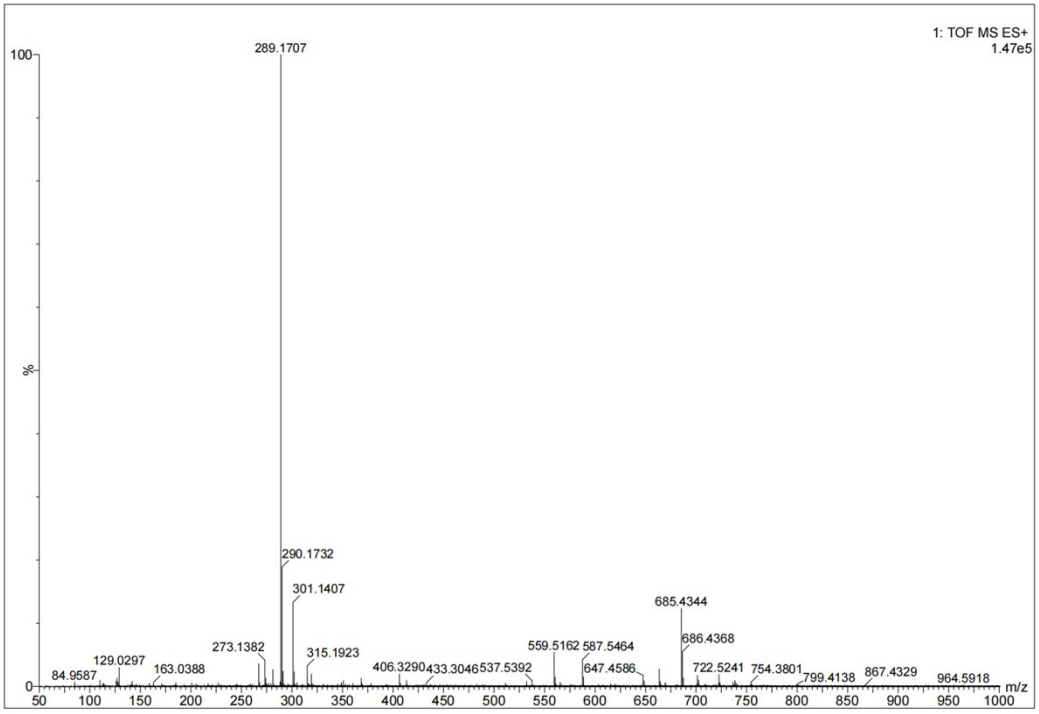


Figure S7. The HRMS spectrum of **MASQ**.

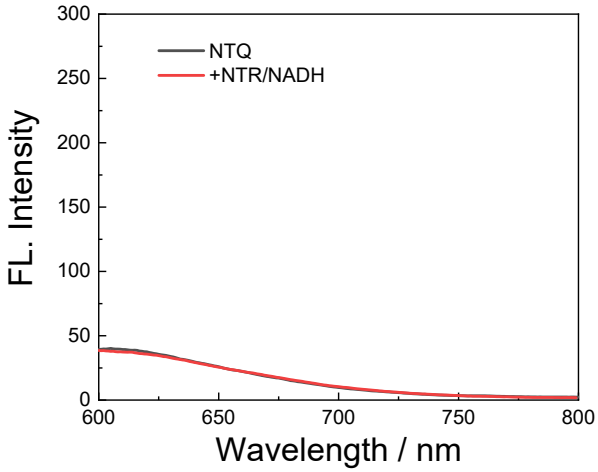


Figure S8. The fluorescence spectra of 10 μM **NTQ** in the absence and presence of nitroreductase (NTR, 1 U) and NADH (500 μM). Excitation wavelength: 561 nm.

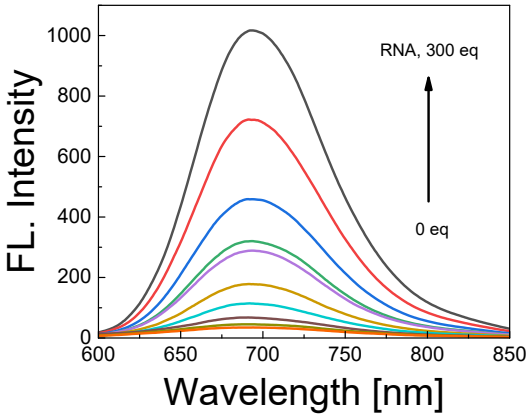


Figure S9. The fluorescence spectra of 5 μM **MASQ** in the presence of 0-300 eq RNA in PBS buffer solution. Excitation wavelength: 560 nm.

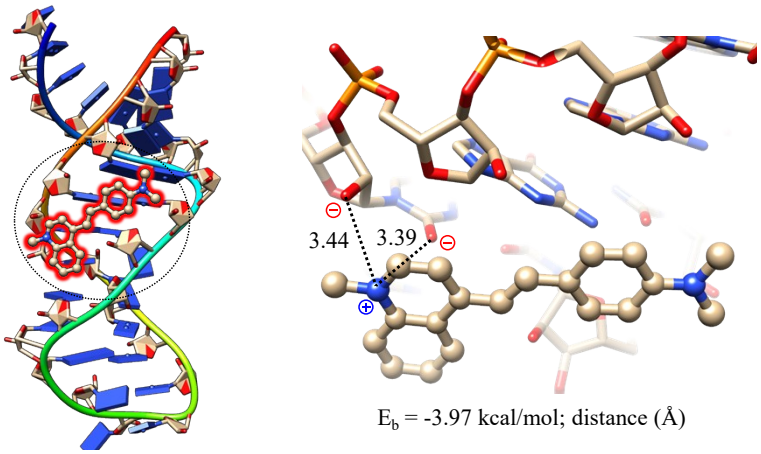


Figure S10. The interaction and binding energy of **MASQ** to RNA calculated by Autodock 4.2 software.

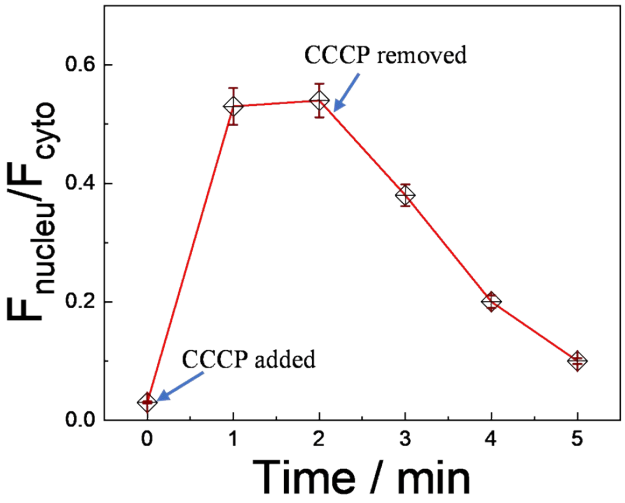


Figure S11. The time-dependent intensity ratio of nuclear to cytoplasm regions in cells loaded with 1 μM **MASQ** for 30 min, treated with 10 μM CCCP for 2 min, and then incubated in fresh culture medium for further 3 min after CCCP removal.

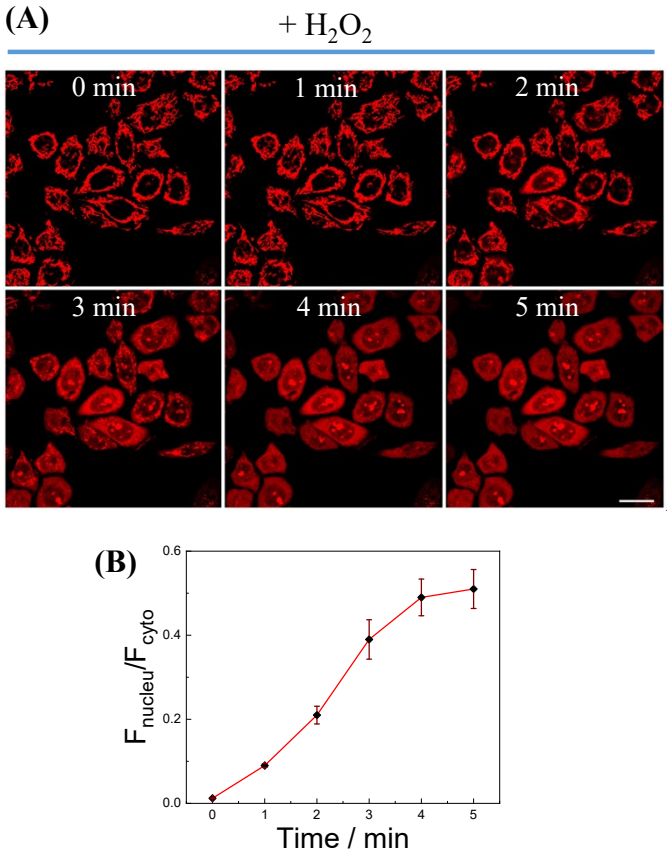


Figure S12. (A) The fluorescence images of live HeLa cells loaded with 1 μM **MASQ** for 30 min, and then incubated in culture medium containing 10 mM H_2O_2 for

5 min. Scale bar = 20 μm. (B) The time-dependent intensity ratio of nuclear to cytoplasm regions in cells loaded with 1 μM **MASQ** for 30 min and then incubated in culture medium containing 10 mM H_2O_2 for 5 min.