Electronic Supplementary Material (ESI) for Chemical Science.

A Dual-Responsive Ratiometric Indicator Designed for in Vivo Monitoring of Oxidative Stress and Antioxidant Capacity

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1.1 Materials and general methods.

Unless otherwise noted, all reagents and chemicals were obtained from commercial suppliers and used without further purification. 4-Methylquinoline was obtained from Aladdin (Shanghai, China). 3-Quinolinecarboxaldehyde, 4-(bromomethyl)-benzeneboronic acid pinacol ester, Methyl trifluoromethanesulphonate (TfOMe), Pyruvic and Piperazine-1,4-bisethanesulfonic acid (PIPES) were from Bide Pharmatech Ltd. (Shanghai, China). β-Nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH) and β-nicotinamide adenine dinucleotide 2'-phosphate reduced tetrasodium salt hydrate (NADPH) were from Macklin (Shanghai, China). Test buffer solutions were obtained by mixing 25 mM PIPES and 100 mM NaCl. The absorbance was recorded by an ultraviolet-visible absorption spectrometer (UV-1900, Shimadzu). Fluorescence spectra were collected on a FS5 fluorescent spectrophotometer (Edinburgh). The NMR-spectra were recorded at room temperature with Bruker Vance III HD 400 MHz spectrometer. The 1H-NMR spectra were recorded at 400 MHz, the 13C-NMR spectra at 100 MHz. The purified water was used in all experiments by a Milli-Q system (Millipore, >18.0 MΩ, USA). All pH measurements were performed with a pH-3C digital pH-meter (Shanghai ShengCi Device Works, Shanghai, China) with a combined glass-calomel electrode. High resolution mass spectrum (HRMS) was measured on a 6540 TOF system (Agilent). Fluorescence imaging in cells, zebrafish and paraffin sections was carried out on a Nikon-Ti2 microscopy imaging system with a 60× immersion objective lens. MDA, LDH, SOD and ATP assay kits were purchased from Beyotime (Shanghai, China).

1.2 Cell Culture.

PC 12 cells were plated in 25 cm^2 cell culture flask in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin. The cells were seeded in confocal culture dishes and then incubated at 100% humidity atmosphere containing 5% CO_2 and 95% air at 37 ^oC.

1.3 MTT assay.

The PC 12 cells were seeded onto 96-well flat-bottomed plates at a density of 1×10^5 cells per well, respectively, and allowed to incubate for 24 hours prior to exposure to varying concentrations of DQ1. Subsequently, 10 µL of the MTT solution (5 mg/mL) was added to each well and kept at 37 °C for 4 h. 100 µL of Formazan solvent was added to each well to dissolve the formed formazan. The microplate reader was used to measure the absorbance at 570 nm after shaking the plates for 4 hours. The following equation was used to calculate the viability of cell growth: Cell viability (%) = (mean value of treatment group-mean value of blank group) / (mean value of control-mean value of blank group) × 100. Each concentration was tested in triplicate, with 5 wells per replicate.

1.4 Colocalization studies.

The co-localization analysis of DQ1 was conducted in PC12 cells using Lyso-Tracker green and Mito-Tracker green. Initially, PC 12 cells were cultured in confocal Petri dishes with bovine albumin culture media. The medium was subsequently aspirated and the cells were washed with 2 mL of PBS per well, repeated three times. Subsequently, PC 12 cells were incubated with 10 µM DQ1 and 100 nM Mito-Tracker Green for a duration of 30 min at a temperature of 37 °C. Colocalization between Lysosome-Tracker Green (50 nM, 30 min) and DAPI (5 µg/ml, 15 min) was performed under the same condition of Mito-Tracker Green. Fluorescent images were acquired using bandpass filters of 600-660 nm upon excitation at 540-580 nm (DQ1), 512-558 nm upon excitation at 465-495 nm (MitoTracker Green), and 432-480 nm upon excitation at 362-396 nm (DAPI). Fluorescent and bright-field images were captured with exposure times of 500 ms and 30 ms, respectively. All images were standardized to the same brightness level using ImageJ (National Institute of Health).

1.5 Fluorescence cells imaging.

Prior to treatment with 10 µM DQ1 in DMEM without fetal bovine serum, cells were seeded on confocal culture dishes and incubated for 24 hours. After a 30-minute incubation period, cells were washed with regular growth medium before imaging. Cellular imaging was performed using a Nikon inverted research microscope, equipped with CFI Plan Apo × 60 oil (NA = 1.4) objective, spinning disk, and a Hamamatsu Orca Flash 4 ms CMOS camera with a bandpass filter of 379/34 nm (Semrock), 456/48 nm (Semrock), 560/40 nm (Semrock) and 630/60 nm (Semrock). All the images were recorded using NIS Elements and then processed using Fiji software. Pearson's correlation coefficients for colocalization were calculated using a Coloc2 Fiji plugin. To demonstrate the capability of DQ1 in detecting intracellular NAD(P)H, PC 12 cells were pre-treated with different concentrations of H_2O_2, glucose, pyruvate, lactate and rotenone, respectively, before subjected to DQ1 treatment. To ensure consistency, the imaging experiments were conducted in triplicate.

1.6 Determination of extracellular release of MDA, LDH, SOD and ATP.

To quantify the secretion of MDA, LDH, SOD and ATP, supernatant from hypoxic cells was collected and extracellular levels of MDA, LDH, SOD and ATP were measured using assay kits in accordance with the manufacturer's instructions.

1.7 Quantification of MDA and ATP release in liver homogenate.

To measure the secretion of MDA and ATP, all mice of different groups were killed after I.V. injection of DQ1 (1 mM, 200 μL) for 12 h. The liver homogenate was collected and extracellular ATP levels were determined using the MDA and ATP Assay Kit following the manufacturer's instructions.

1.8 Zebrafish embryo imaging experiment.

In the hypoxia experiments, zebrafish were cultured in incubators with approximately 1% O_2 and divided into four groups based on different incubation time intervals (0, 1, 2, 3 h). Subsequently, the zebrafish were incubated with DQ1 (20 μM) at 28.5 °C for a duration of 30 minutes. Prior to DQ1 treatment, zebrafish were preincubated with either 20 or 50 μM H_2O_2 for 1 hour or 2 or 5 μM retonone for 2 hours in the stimulation experiments. Subsequently, the zebrafish were subjected to PBS washing, paraformaldehyde fixation, and imaging using a Nikon inverted research microscope. Fluorescence intensity was analyzed using ImageJ software. Blue channel: λ_{ex} = 362-396 nm, λ_{em} = 432-480 nm; Red channel: λ_{ex} = 540-580 nm, λ_{em} = 600-660 nm.

1.9 Fluorescence imaging of paraffin-embedded tissue sections.

The mouse infarction model was established through ligation of the left descending coronary artery, and the mice were divided into four groups based on the duration of infarction: 1, 3, 5, and 7 days. Normal unoperated mice were used as controls. At 24 hours post-surgery, the hearts were extracted and probe DQ1 (1 mM, 200 μL) was administered intravenously through the tail vein. After a duration of twelve hours, the hearts were excised for analysis. The extent of infarcted tissue was determined using the TTC method. The hearts of mice were cryopreserved for 20 minutes and subsequently cross-sectioned into 2-3 mm thick slices within the infarcted region. These sections were stained with TTC (2%). The remaining tissue was further sectioned and imaged using fluorescence microscopy. procedures were conducted in accordance with the regulations established by the Ethical Committee of Nantong University.

1.10 Synthesis and characterization of DQ1.

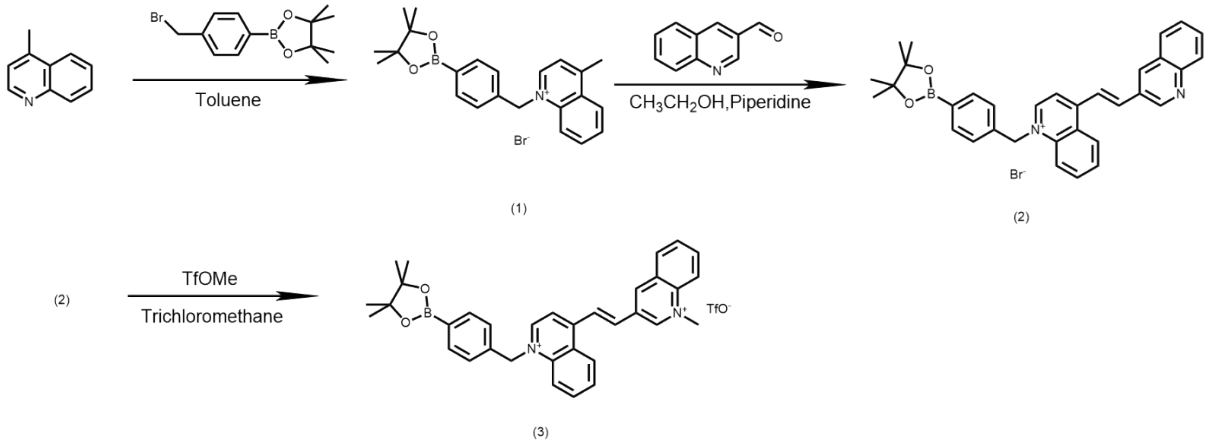


Figure S1. The synthetic procedure used to prepare probe DQ1.

Compound 1. A mixture of 4-Methylquinoline (1.00 g, 6.99 mmol) and (4-Bromomethylphenyl) boronic acid (2.49 g, 8.33 mmol) was dissolved in toluene (20 mL) and refluxed at 110 °C for 18 hours. The mixture was then cooled to room temperature and concentrated in vacuum. The crude product was filtered upon the addition of 50 mL ether. The resulting precipitate was collected via suction filtration followed by extensive washing with ether to obtain an off-white solid (0.72 g, 30.1% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.66 - 9.60 (d, J = 6.0 Hz, 1H), 8.60 - 8.53 (dd, J = 8.5, 1.4 Hz, 1H), 8.42 - 8.35 (d, J = 8.9 Hz, 1H), 8.23 - 8.17 (d, J = 6.1 Hz, 1H), 8.21 – 8.12 (ddd, J = 8.7, 6.9, 1.4 Hz, 1H), 8.06 - 7.98 (m, 1H), 7.70 - 7.63 (m, 2H), 7.37 - 7.31 (d, J = 7.8 Hz, 2H), 6.39 - 6.34 (s, 2H), 3.08 - 3.04 (s, 3H), 1.29 - 1.24 (s, 12H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 160.31, 149.97, 137.93, 137.38, 135.81, 135.60, 130.27, 129.73, 127.93, 127.04, 123.58, 120.25, 84.38, 60.18, 25.20, 20.44.

Compound 2. To a mixture of compound 1 (0.50 g, 1.13 mmol) and 3-Quinolinecarboxaldehyde (0.35 g, 2.26 mmol) in 20 mL anhydrous ethanol was added piperidine (20 μL). Then the mixture was refluxed for 18 h under a nitrogen atmosphere. After cooling down to room temperature, the precipitate was filtered, washed with cold ether, and dried in vacuum to obtain compound 2 as a green solid (0.55 g, 81.9% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.81 - 9.77 (s, 1H), 9.77 - 9.71 (d, J = 6.5 Hz, 1H), 9.60 - 9.55 (d, J = 2.1 Hz, 1H), 9.21 - 9.15 (d, J = 8.7 Hz, 1H), 9.02 - 8.91 (d, J = 2.1 Hz, 1H), 8.78 - 8.67 (m, 2H), 8.51 - 8.44 (d, J = 16.1 Hz, 1H), 8.44 - 8.36 (d, J = 9.0 Hz, 1H), 8.24 - 8.13 (m, 1H), 8.16 - 8.10 (d, J = 4.7 Hz, 1H), 8.13 - 8.08 (s, 1H), 8.10 - 7.96 (m, 1H), 7.92 - 7.83 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H), 7.83 - 7.64 (m, 3H), 7.64 - 7.44 (qd, J = 17.9, 15.9, 8.0 Hz, 1H), 7.42 - 7.38 (s, 1H), 7.40 - 7.29 (m, 1H), 6.41 - 6.33 (s, 2H), 1.29 - 1.25 (s, 12H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 153.81, 151.04, 149.48, 148.56, 140.99, 138.52, 138.14, 136.73, 135.69, 131.68, 130.18, 129.57, 129.49, 129.25, 128.30, 127.94, 127.72, 127.03, 122.60, 84.40, 44.27, 25.26, 22.73, 22.19.

Compound 3. Methyl trifluoromethanesulphonate (TfOMe, 0.45 g, 2.76 mmol) was added to a suspension of compound 2 (0.3 g, 0.55 mmol) in dry DCM (15 mL) at room temperature under the nitrogen atmosphere. The mixture was stirred for 8 h. Then the precipitate was filtered off, washed with DCM and ether, and dried in vacuo to afford pure DQ1 as a chartreuse powder (0.35 g, 70.5%).
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.09 - 10.02 (d, J = 1.8 Hz, 1H), 9.69 - 9.63 (s, 1H), 9.57 - 9.50 (d, J = 6.4 Hz, 1H), 9.09 - 9.02 (d, J = 7.3 Hz, 1H), 8.84 - 8.73 (d, J = 16.3 Hz, 1H), 8.64 - 8.56 (m, 2H), 8.56 - 8.46 (m, 2H), 8.41 - 8.30 (m, 2H), 8.30 - 8.20 (m, 2H), 8.18 - 8.11 (m, 2H), 7.86 - 7.78 (d, J = 8.0 Hz, 2H), 7.42 - 7.34 (d, J = 7.8 Hz, 2H), 6.42 - 6.35 (d, J = 7.6 Hz, 2H), 4.88 - 4.81 (s, 3H), 1.41 - 1.28 (s, 12H).
^{13}C NMR (100 MHz, DMSO-d_6) δ 152.48, 150.44, 149.89, 145.43, 138.51, 138.37, 137.82, 136.64, 136.42, 136.12, 135.57, 131.41, 131.21, 130.36, 129.88, 129.39, 127.84, 127.06, 125.90, 122.73, 120.40, 119.97, 119.52, 118.50, 84.33, 65.40, 60.29, 46.39, 25.42, 25.09.
MALDA-TOF/MS (ESI) m/z: calcd for $[C_{35}H_{35}BF_3N_2O_5S]^+$ 663.23, found 663.23.

1.11 Preparation of the test solution.

The stock solution of DQ1 (1 mM) was prepared in DMSO. The UV/Vis absorption and fluorescence responses of DQ1 to NADH were measured in PIPES buffer solutions (25 mM PIPES, 100 mM NaCl, pH 7.4). NADH stock solution (500 μM) was prepared in PB buffer solutions (50 mM, pH = 7.4).

1.12 Determination of the detection limit.

The limit of detection (LOD) was determined by fluorescence titration of the DQ1 (20 μM) in the presence of varying concentrations of H_2O_2 (0-300 μM) and NADH (0-30 μM). The fluorescence emission spectrum of DQ1 was measured and the standard deviation of the blank measurement was obtained. The detection limit was calculated using the following equation:

$$LOD=3\sigma/k$$

Where σ is the standard deviation of the blank measurement, k is the slope between the fluorescence intensity (F505 nm) versus H_2O_2 concentrations and (F650 nm) versus NADH concentrations.

1.13 The hematoxylin and eosin (H&E) staining assays.

Initially, the heart and liver tissues were sectioned into consecutive paraffin histological sections. The paraffin sections were subjected to a 30-minute xylene deparaffinization process, followed by an 8-minute hematoxylin staining procedure. After washing with water, all the sections were immersed in hydrochloric acid 0.5% (v/v) for 30 seconds. Wash the sections with water for 1 min, followed by bluing in 0.2% ammonia water for 30 seconds. Afterward, counterstain the sections in eosin Y solution for 30 seconds. Finally, the sections were ready to use by dehydrating through 95% alcohol for 10 mins.

1.14 Statistical Analysis.

The experiments were performed at least three times, and all data were presented as mean ± standard deviation (SD). The figures presented in this article were derived from three separate experiments, all of which produced consistent results unless otherwise indicated. The statistical analysis of the differences between two groups was conducted using a two-tailed Student's t-test. A significance level of $P < 0.05$ (*) or $P < 0.01$ (**) was considered statistically significant.

2.1 HPLC chromatographs of compound 2 and DQ1.

Time (min)	Flow rates mL/min.	A % (H_2O)	B% (MeOH)
0	0.8	50	50
5	0.8	40	60
5.01	0.8	20	80
15	0.8	20	80
15.01	0.8	0	100
30	0.8	0	100

Gradient conditions for compound 2 purification. Monitored at 400 nm wavelength.

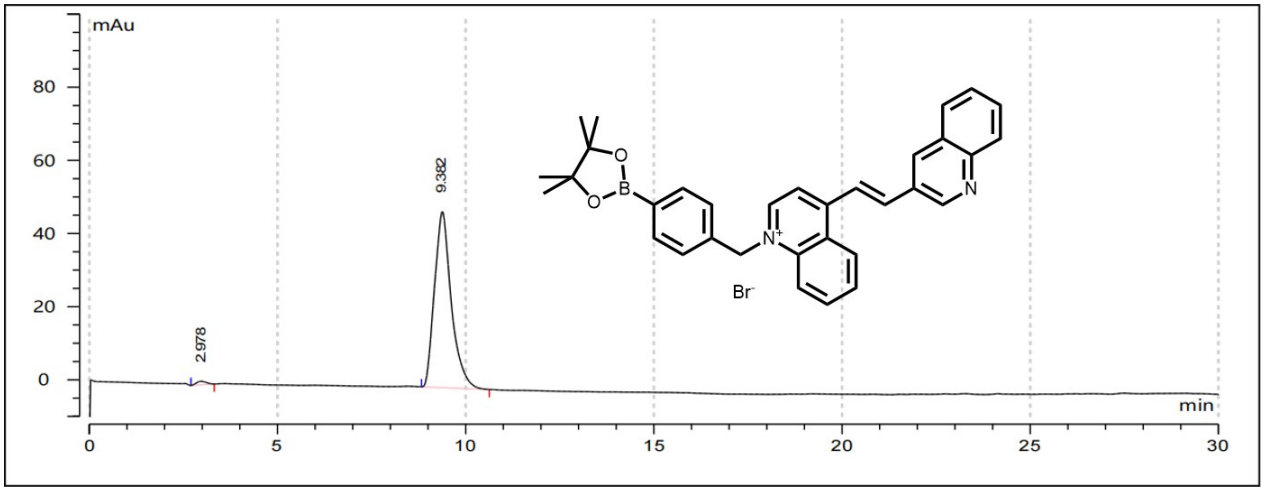


Figure S2 HPLC chromatographs of compound 2.

No	retention time (min)	peak area (mAu*s)	peak width (min)	FWHM (min)	peak height (mAu)	peak area ratio (%)
1	2.978	45.79058	0.720	0.407	1.063	2.986
2	9.382	1487.82851	1.801	0.463	48.326	97.014

Time (min)	Flow rates mL/min.	A % (H_2O)	B% (MeOH)
0	0.8	20	80
1	0.8	20	80
5.01	0.8	10	90
10	0.8	10	90
10.01	0.8	0	100
30	0.8	0	100

Gradient conditions for DQ1 purification. Monitored at 370 nm wavelength.

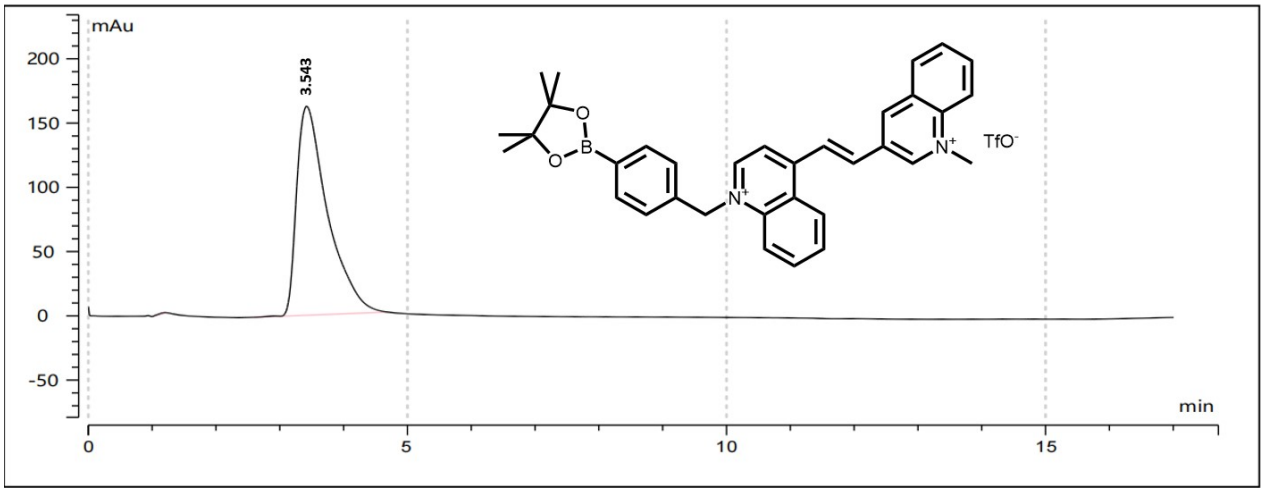


Figure S3 HPLC chromatographs of DQ1.

No	retention time (min)	peak area (mAu*s)	peak width (min)	FWHM (min)	peak height (mAu)	peak area ratio (%)
1	1.197	5.38080	0.205	0.172	0.438	0.098
2	2.945	5470.42723	2.085	1.904	0.383	99.902

2.2 ^{1}H NMR and ^{13}C NMR spectra of DQ1 in DMSO-d_6

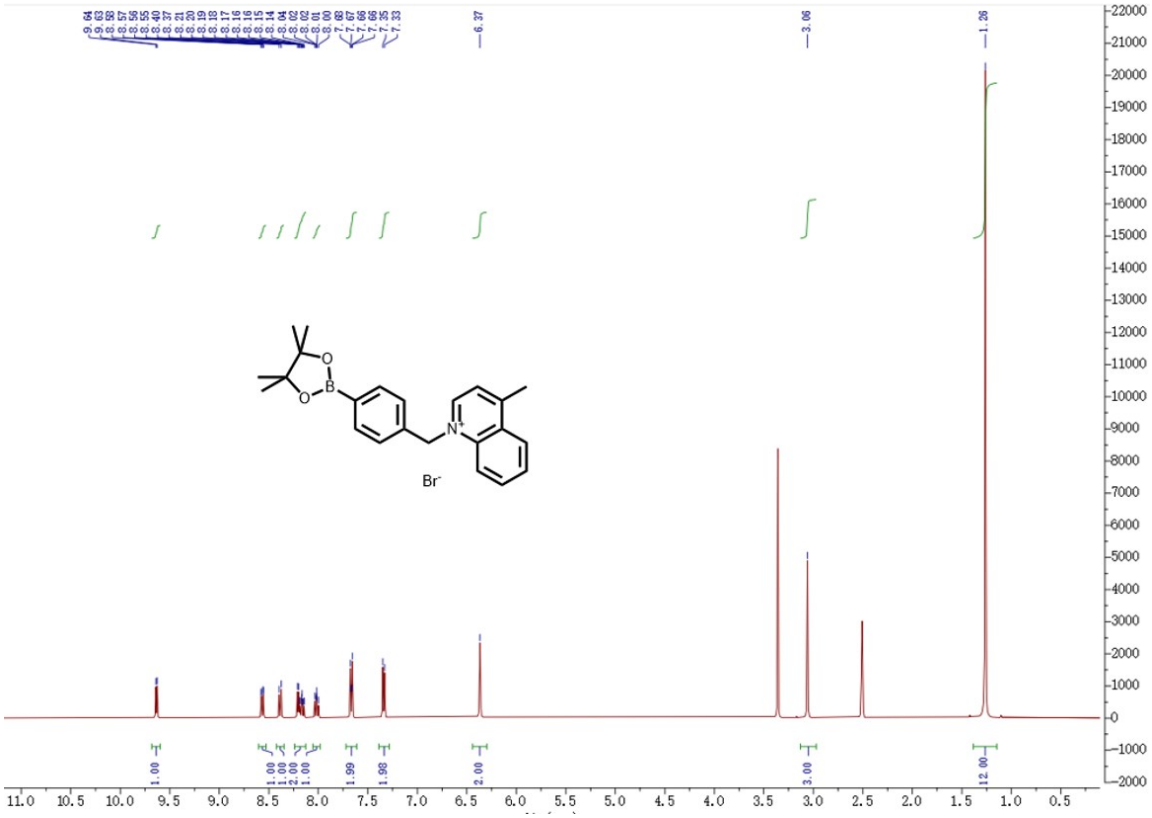


Figure S4. ^{1}H NMR spectrum (400 MHz, **DMSO-d_6**) of compound 1.

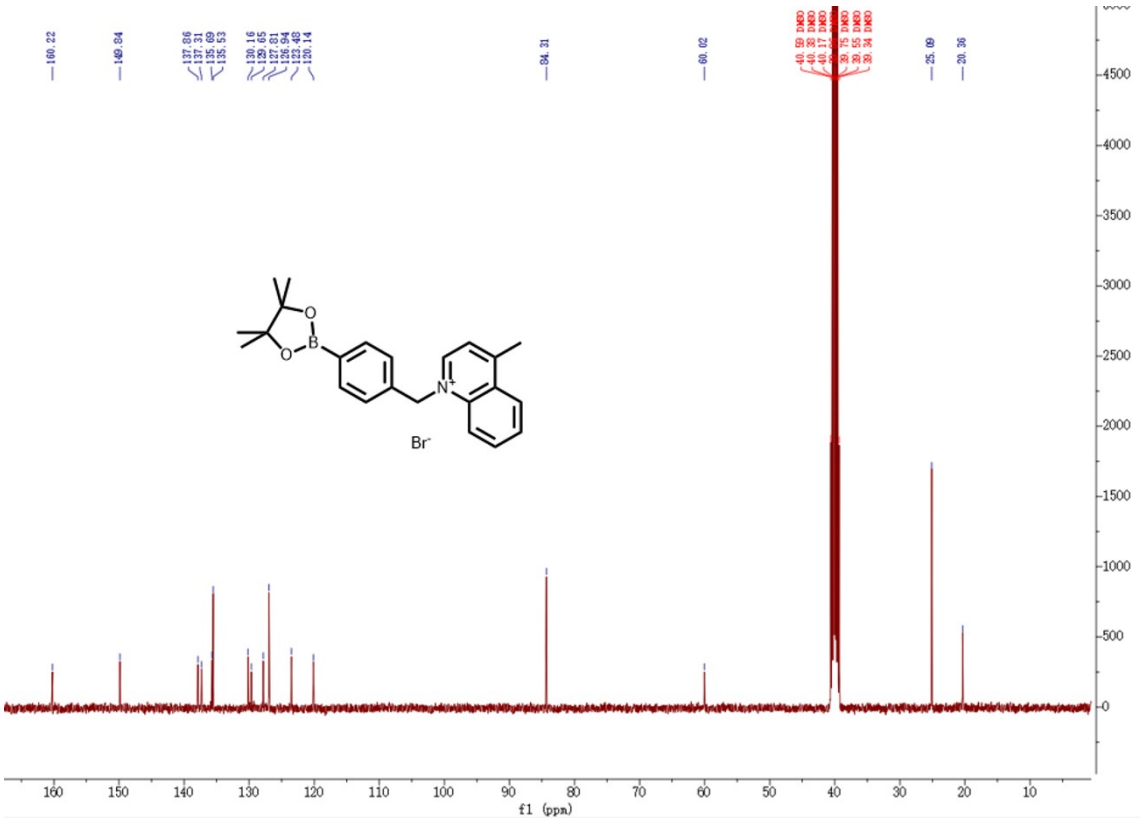


Figure S5. ^{13}C NMR spectrum (100 MHz, **DMSO-d_6**) of compound 1.

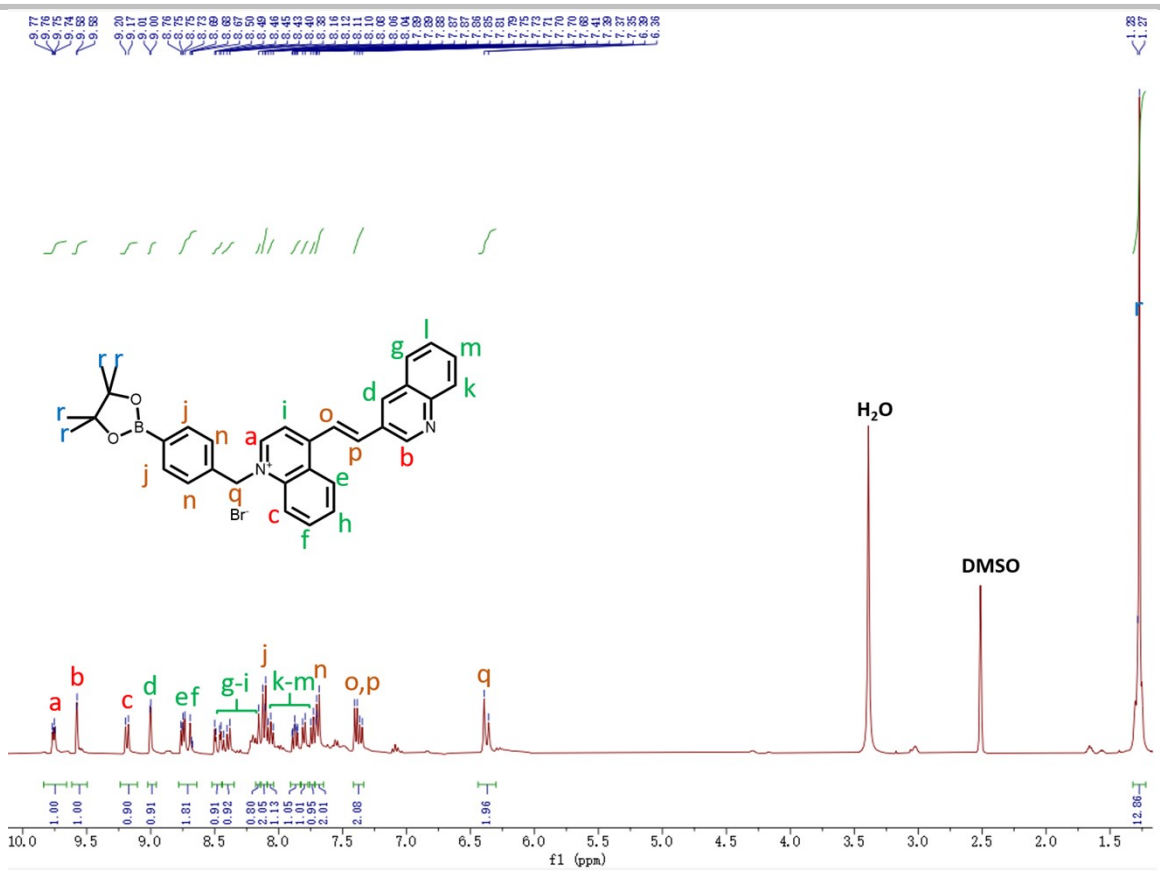


Figure S6. ^{1}H NMR spectrum (400 MHz, **DMSO-d_6**) of compound 2.

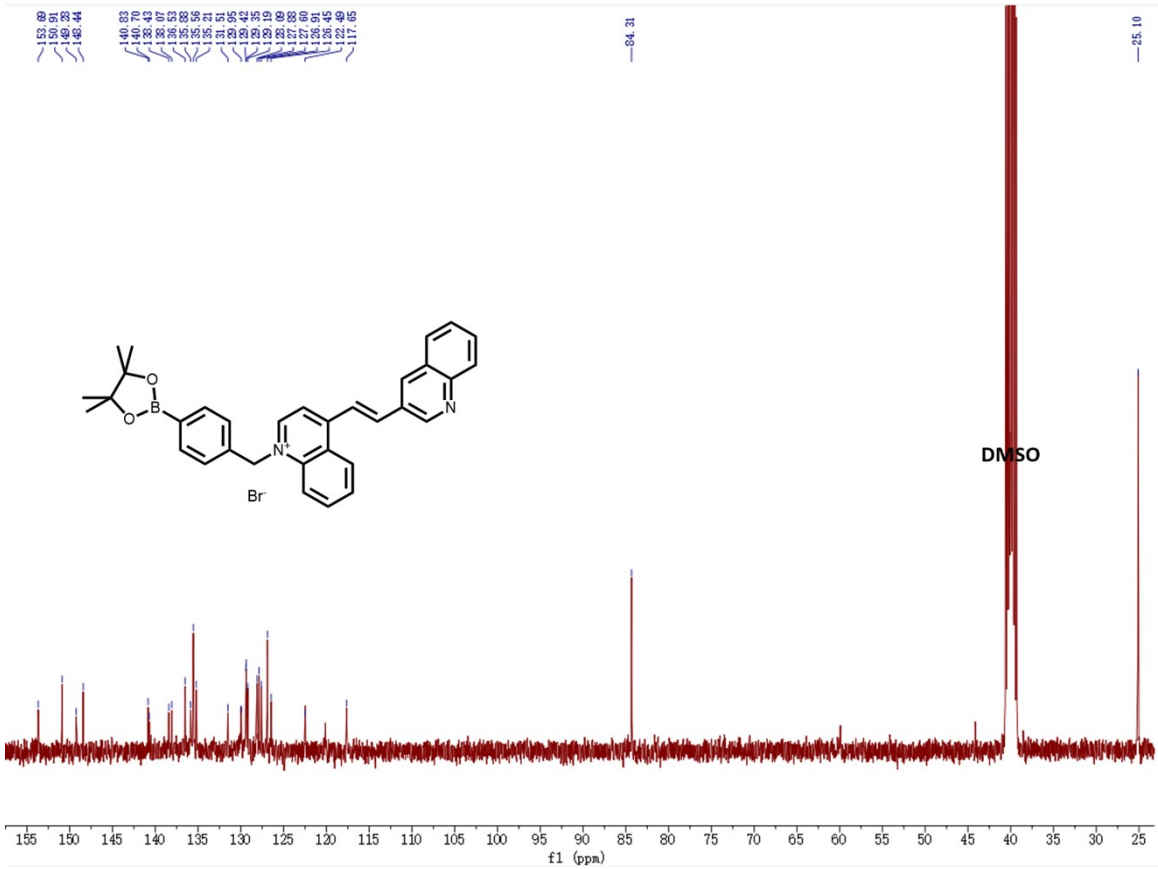


Figure S7. ^{13}C NMR spectrum (100 MHz, **DMSO-d_6**) of compound 2.

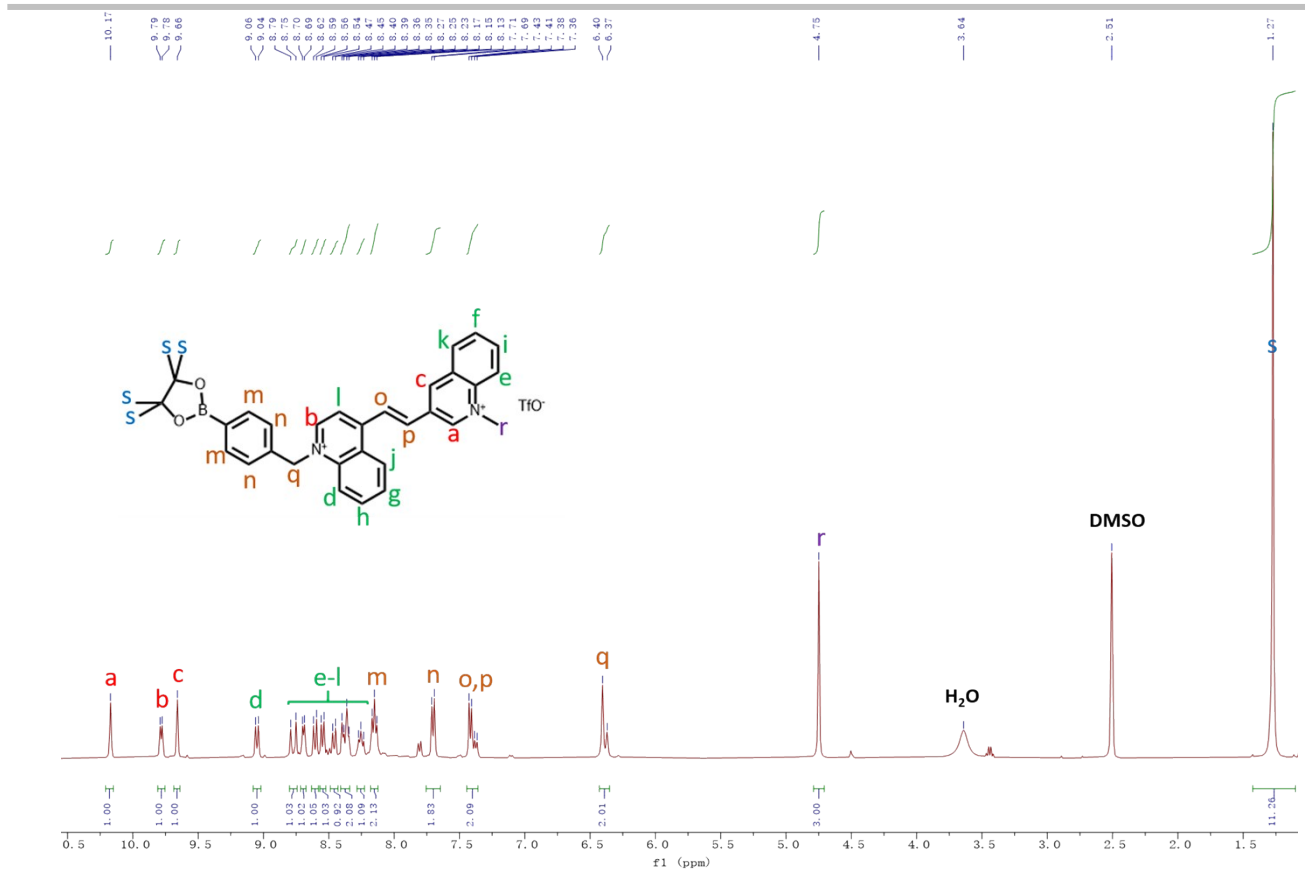


Figure S8. ^{1}H NMR spectrum (400 MHz, **DMSO-d_6**) of compound DQ1.

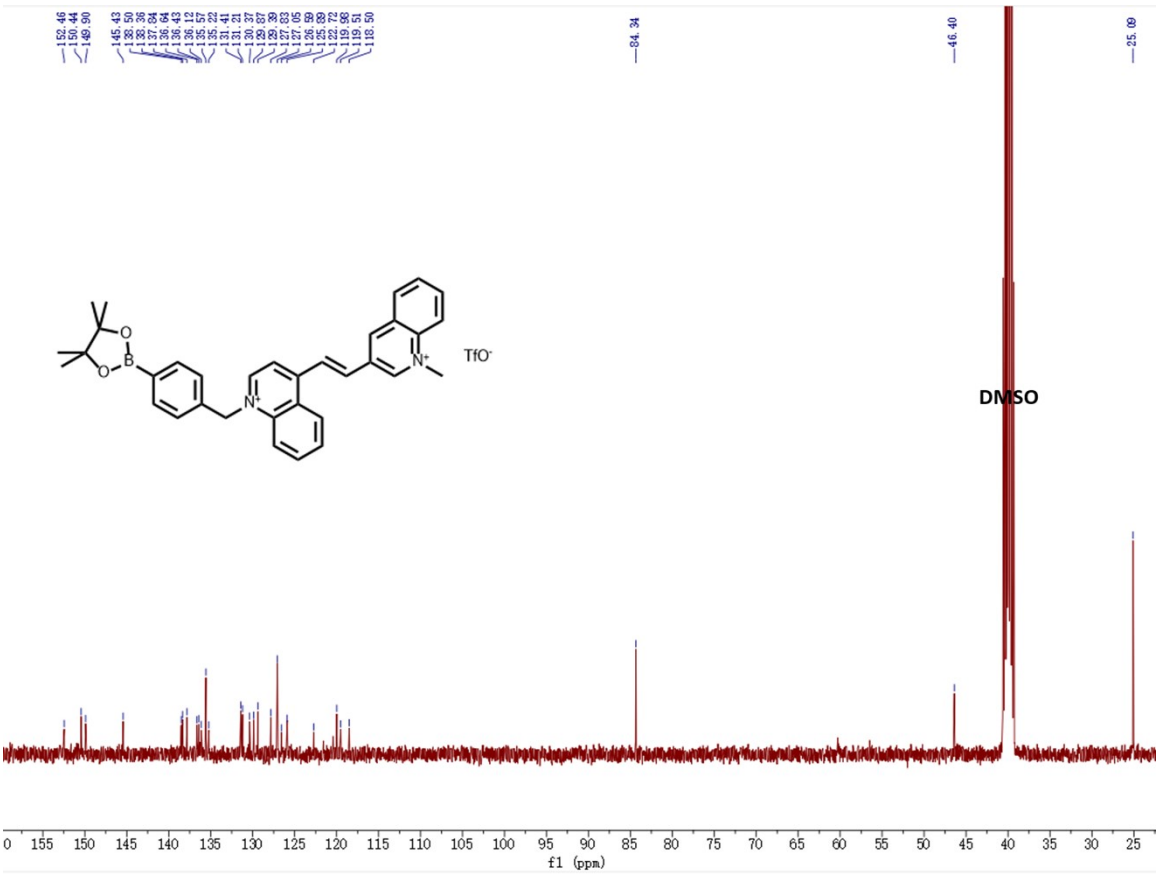


Figure S9. ^{13}C NMR spectrum (100 MHz, **DMSO-d_6**) of compound DQ1.

2.3 MALDI-TOF/MS for DQ1

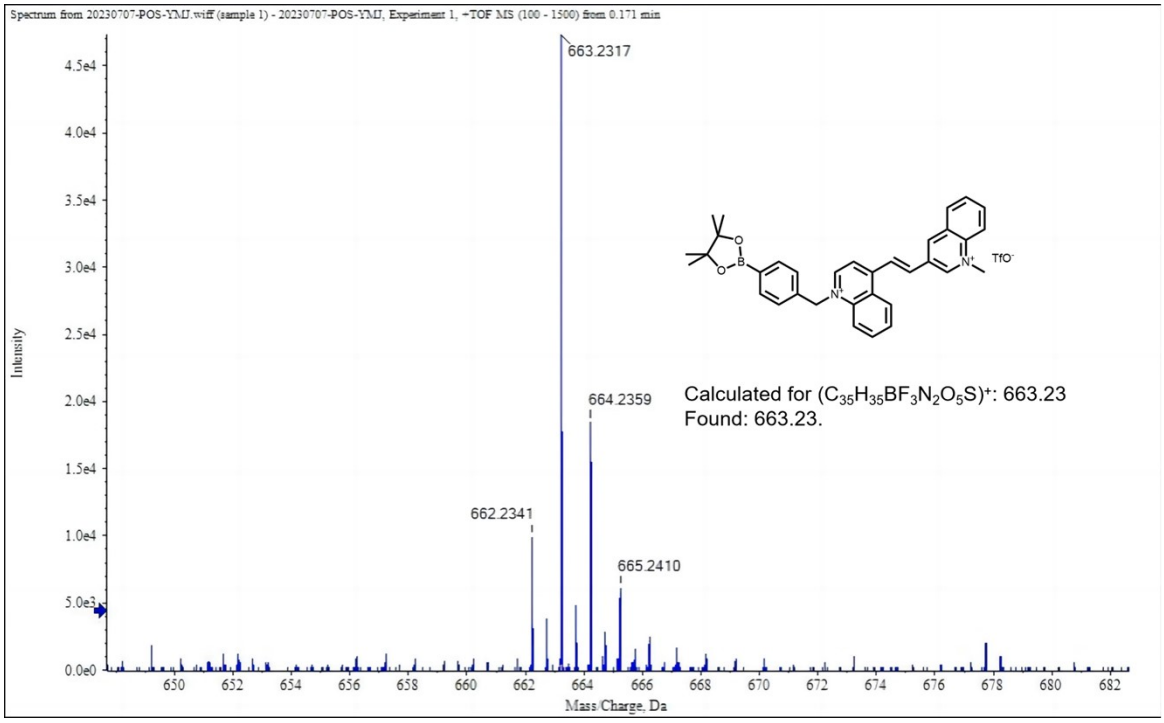


Figure S10. MALDI-TOF/MS for DQ1.

2.4 The absorption and emission spectra of DQ1 in the presence of both H_2O_2 and NADH

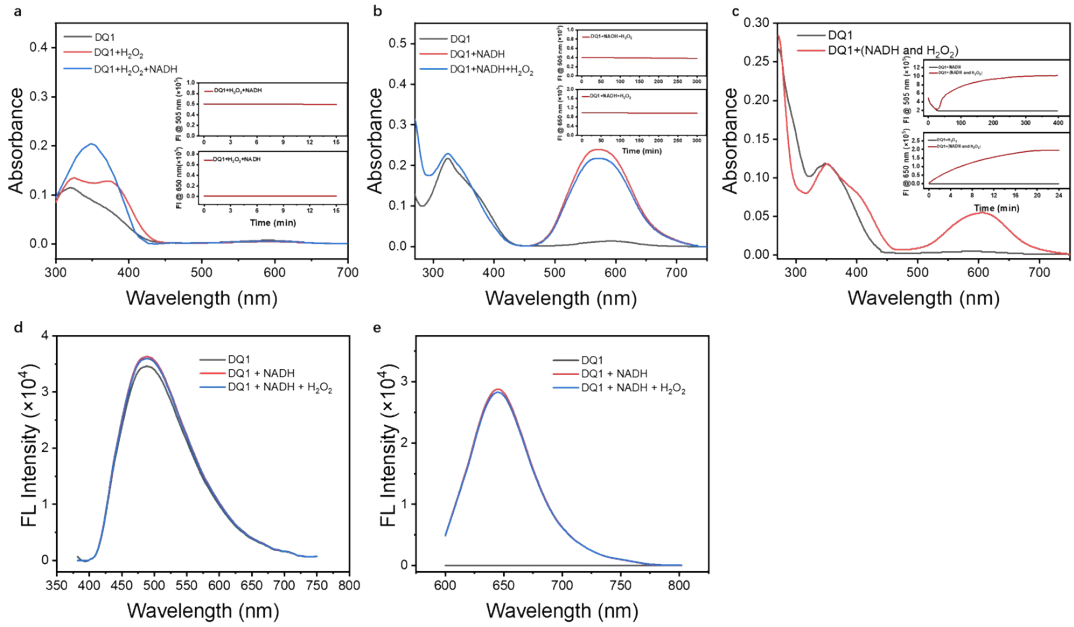


Figure S11. (a) The absorption spectra of DQ1 during consecutive reactions with H_2O_2 and NADH. Inset: Fluorescence intensity change curves at 505 nm and 650 nm. (b) The absorption spectra of DQ1 during sequential reactions with NADH and H_2O_2. Inset: Fluorescence intensity change curves at 505 nm and 650 nm. (c) The absorption spectra of DQ1 upon simultaneous reaction with H_2O_2 and NADH. Inset: The fluorescence intensity changing curves at 505 nm and 650 nm. (d) Fluorescence spectra of DQ1 after reaction with NADH and H_2O_2 excited at 370 nm. (e) Fluorescence spectra of DQ1 after reaction with NADH and H_2O_2 excited at 590 nm.

2.5 The pH-Dependent optical properties of DQ1

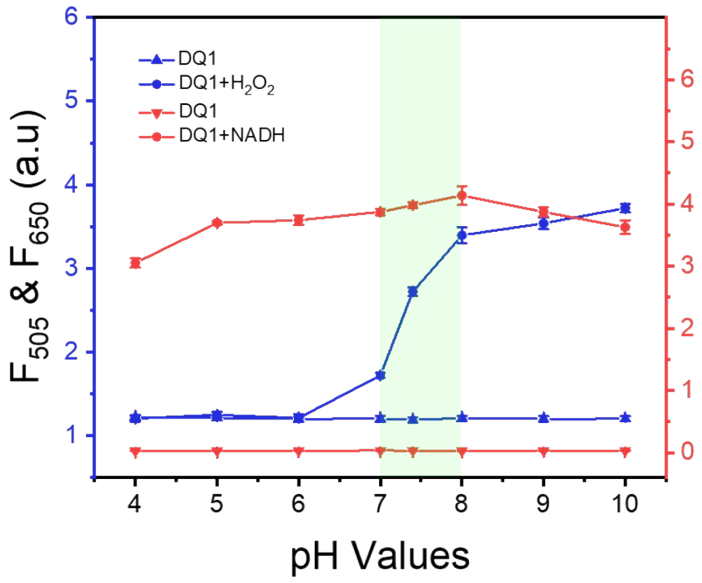


Figure S12. Blue lines: The pH-dependent fluorescence changes of DQ1 (20 μM, 3h) in the absence and presence of H_2O_2 (300 μM). Measurement condition: 25 mM PIPES buffer saline solution, 37 °C, λ_{ex} = 370 nm. Red lines: The pH-dependent fluorescence changes of DQ1 (20 μM, 15 min) in the absence and presence of NADH (24 μM). Measurement condition: 25 mM PIPES buffer saline solution, 37 °C, λ_{ex} = 590 nm. Error bars represent standard deviation (n = 3).

2.6 The time-dependent fluorescence intensity and interference test of DQ1

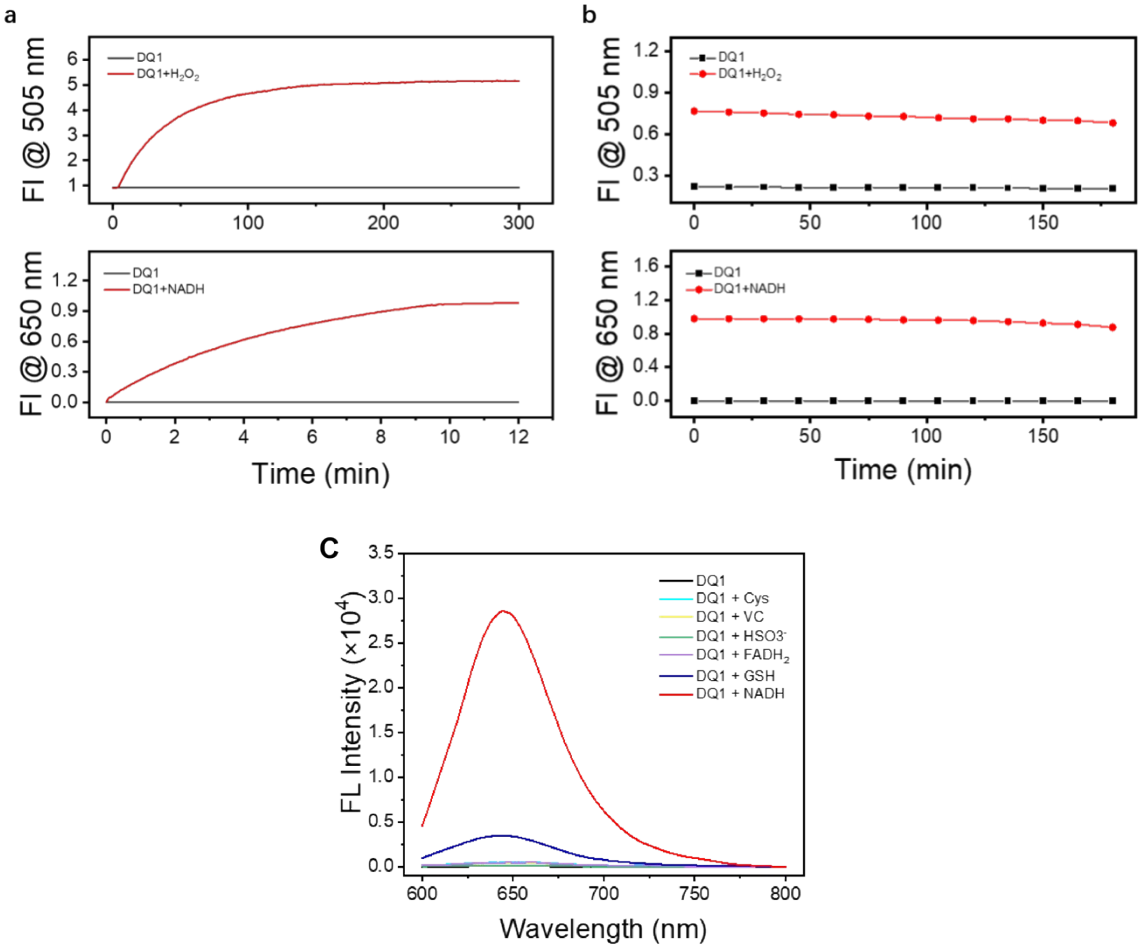


Figure S13. (a) Time-dependent fluorescence intensity of DQ1 (20 μM) at 505 nm and 650 nm in the absence (black) and presence (red) of H_2O_2 (300 μM), NADH (30 μM) respectively. All data were recorded in PIPES buffer (25 mM, pH = 7.4) at 37 °C. (b) Photostability evaluation of DQ1 under UV irradiation. DQ1 (10 mM) was dissolved in PBS solution (pH = 7.4) containing 1% DMSO. Fluorescence measurements were recorded at room temperature for 3 h. (c) Fluorescence spectra of DQ1 with the addition of different substances (20 μM) for NADH detection (Cys, Ascorbic acid (VC); HSO_3^-, $FADH_2$, GSH, NADH). λ_{ex}: 590 nm, each datum was acquired after various analytes addition at 37 °C.

2.7 Cytotoxicity assays

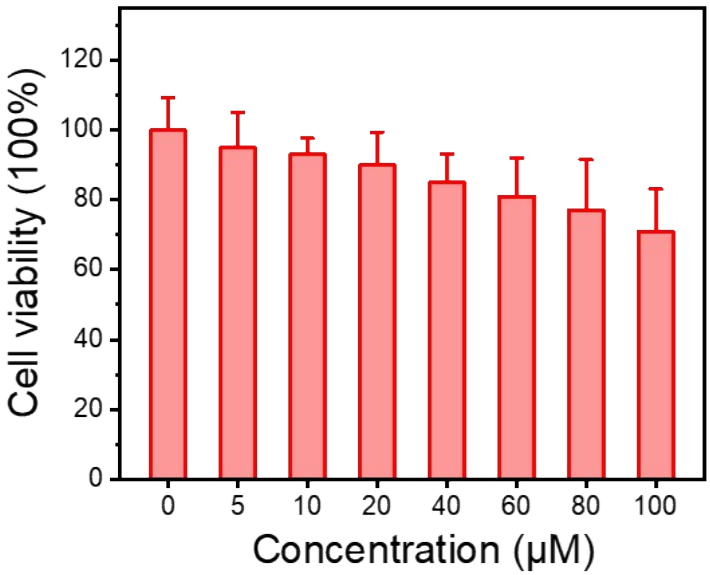


Figure S14. The PC12 cells viability after incubation with various concentrations of DQ-1 (0, 5, 10, 20, 40, 60, 80 and 100 μM) for 24 h. The viability of PC12 cells without DQ1 is defined as 100%. The results represent the mean ± standard deviation of five independent measurements.

2.8 Fluorescence colocalization microscopy analysis of DQ1

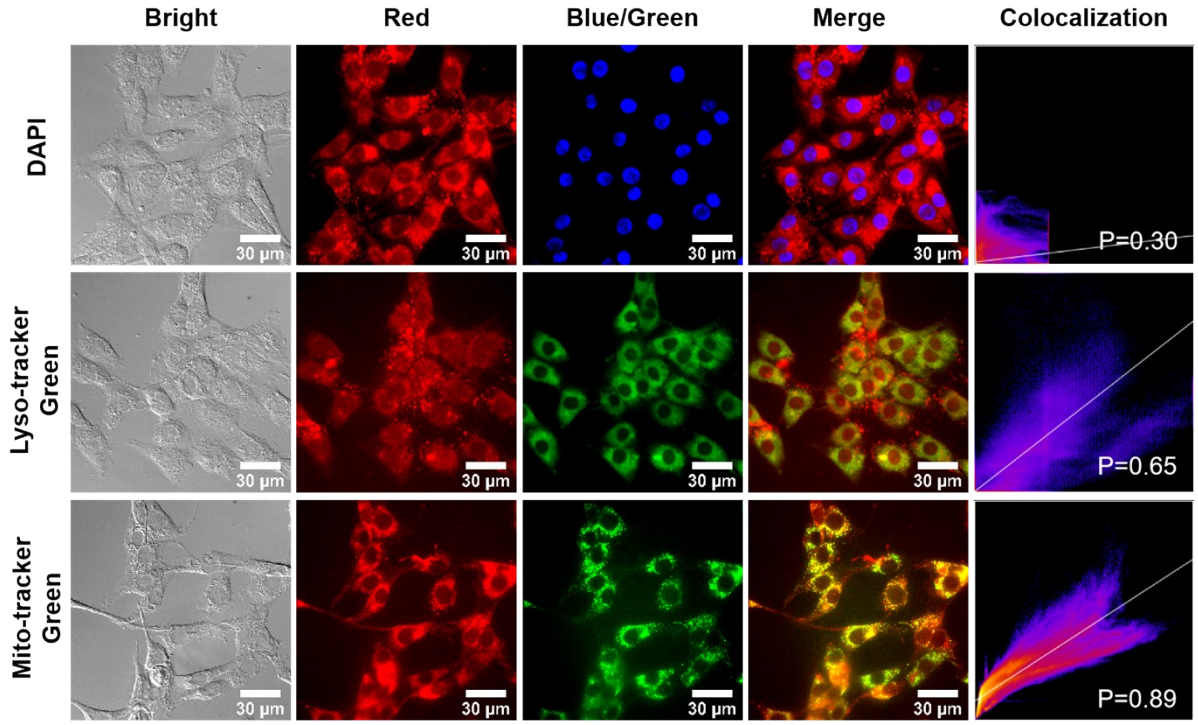
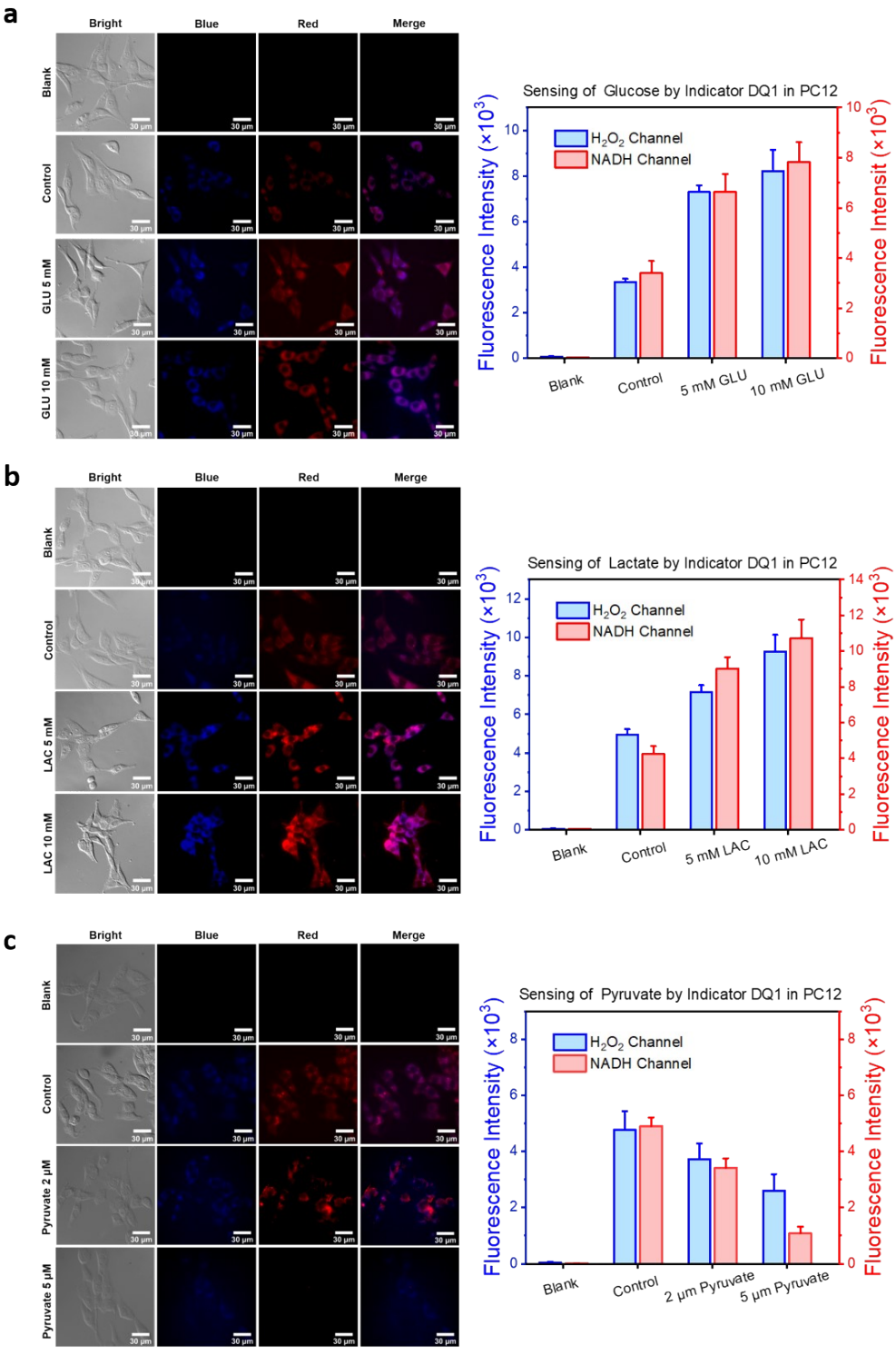


Figure S15. Fluorescence microscopic images of PC12 cells stained with probe DQ1 (10 μM, 30 min) and Lyso-Tracker green (50 nM, 30 min), DAPI (5 μg/ml, 15 min), Mito-Tracker green (100 nM, 30 min). Red channel, λ_{ex}: 525-570 nm λ_{em}: 570-640 nm, green channel, λ_{ex}: 465-495 nm, λ_{em}: 512-588 nm, blue channel, λ_{ex}: 362-396 nm, λ_{em}: 432-480 nm. Scale bar: 30 μm.

2.9 Detection of H_2O_2 and NAD(P)H in PC12 cells through stimulation with H_2O_2, glucose, lactate, pyruvate, rotenone and NADH

To demonstrate the capability of DQ1 in detecting intracellular H_2O_2 and NAD(P)H, PC12 cells were exposed to varying concentrations of H_2O_2, glucose, pyruvate, lactate, rotenone and NADH.



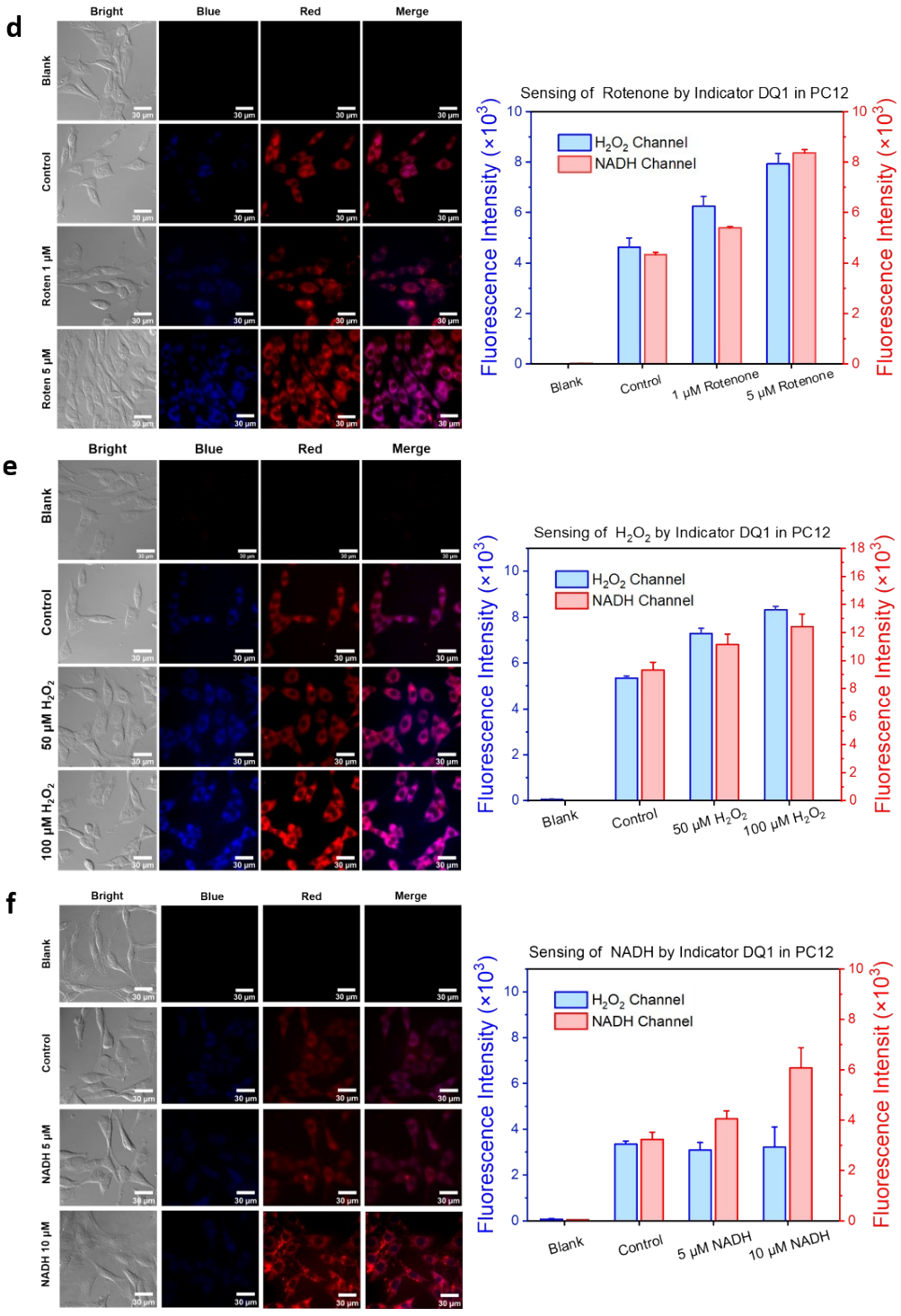


Figure S16. Fluorescence, bright field images of PC12 cells and their corresponding mean fluorescence intensities of blue and red channels. (a) 5 mM and 10 mM glucose pretreated PC 12 cells stained with DQ1 (20 μM, 30 min). (b) 5 mM and 10 mM lactate pretreated PC 12 cells stained with DQ1 (20 μM, 30 min). (c) 2 μM and 5 μM pyruvicacid pretreated PC 12 cells stained with DQ1 (20 μM, 30 min). (d) 1 μM and 5 μM rotenone pretreated PC 12 cells stained with DQ1 (20 μM, 30 min). (e) 50 μM and 100 μM H_2O_2 pretreated PC 12 cells stained with DQ1 (20 μM, 30 min). (f) 5 μM and 10 μM NADH pretreated PC 12 cells stained with DQ1 (20 μM, 30 min). Red channel, λ_{ex}: 540-580 nm λ_{em}: 600-660 nm, blue channel, λ_{ex}: 362-396 nm, λ_{em}: 432-480 nm. Scale bar: 30 μm.

2.10 Reagent kit assay of hypoxic PC 12 cells

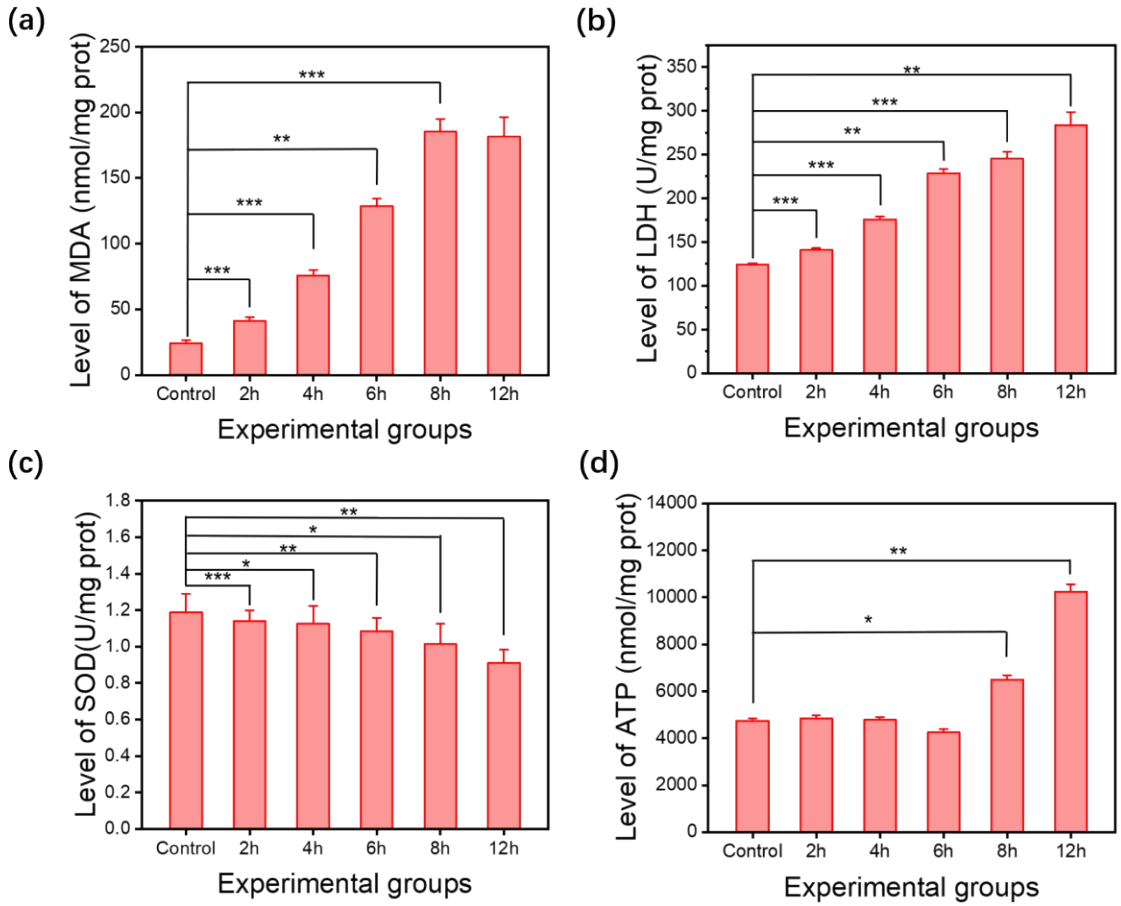


Figure S17. (a) Changes in cellular MDA content after acute hypoxia. (b) Changes in cellular LDH content after acute hypoxia. (c) Changes in cellular SOD content after acute hypoxia. (a) Changes in cellular ATP content after acute hypoxia. (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$)

2.11 TTC staining pictures of myocardial infarction tissues from ischemic mouse hearts

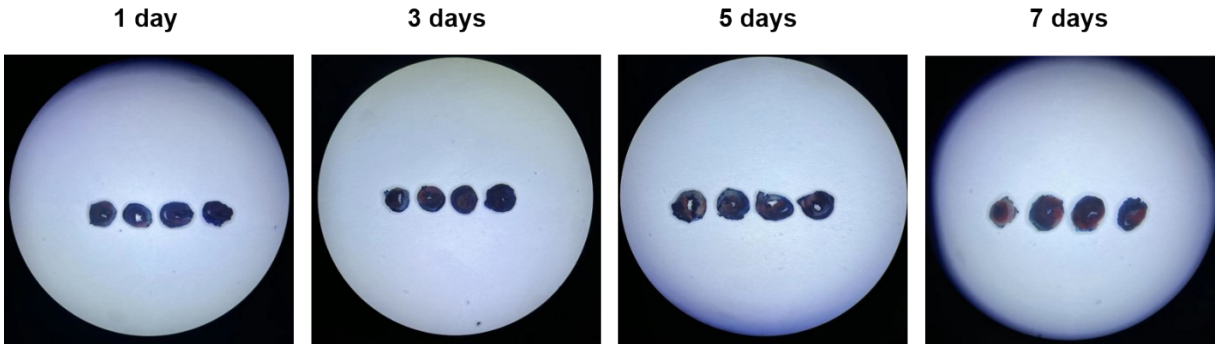


Figure S18. 2,3,5-triphenyltetrazolium chloride (TTC) (2%) stained mouse heart tissues at different time stages following acute myocardial infarction (1, 3, 5, 7 days).

2.12 H&E-stained tissue sections of different organs after TCE exposure

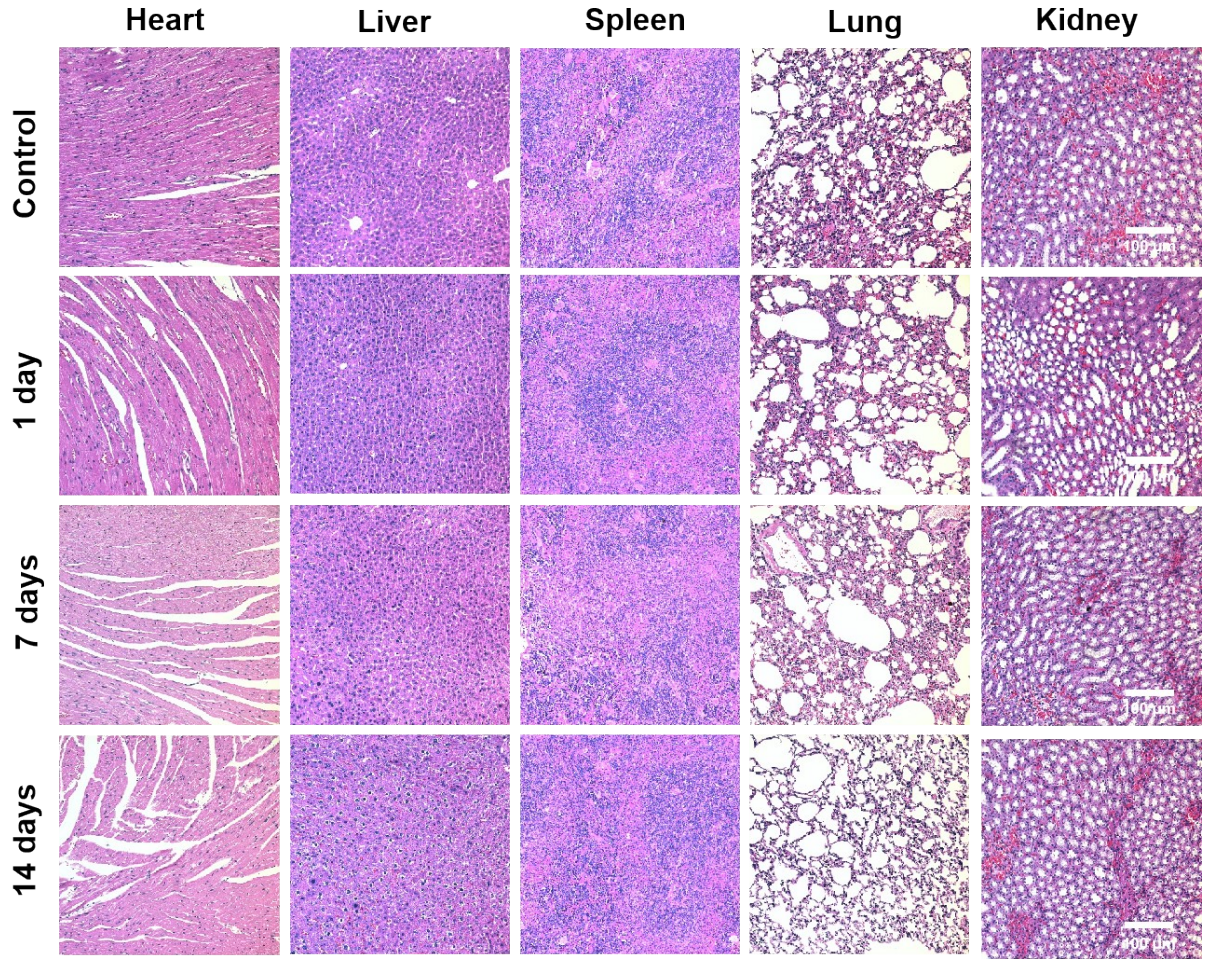


Figure S19. H&E-stained tissue sections of the heart, liver, spleen, lung and kidney harvested from mice after intravenous injection of TCE without (control) or with DQ1 (1mM, 200 μL). Scale bar: 100 μm.

2.13 Image of H&E-stained tissue sections from ischemic mouse hearts

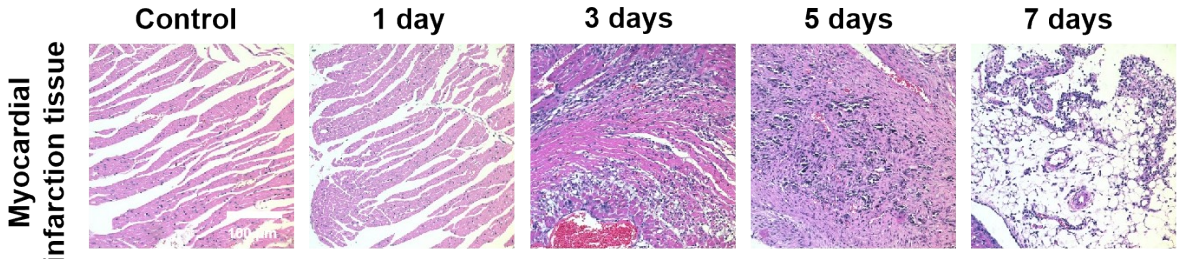


Figure S20. H&E-stained tissue sections of infarcted mouse hearts.

2.14 H&E and DQ1-stained histological analysis of heart tissues

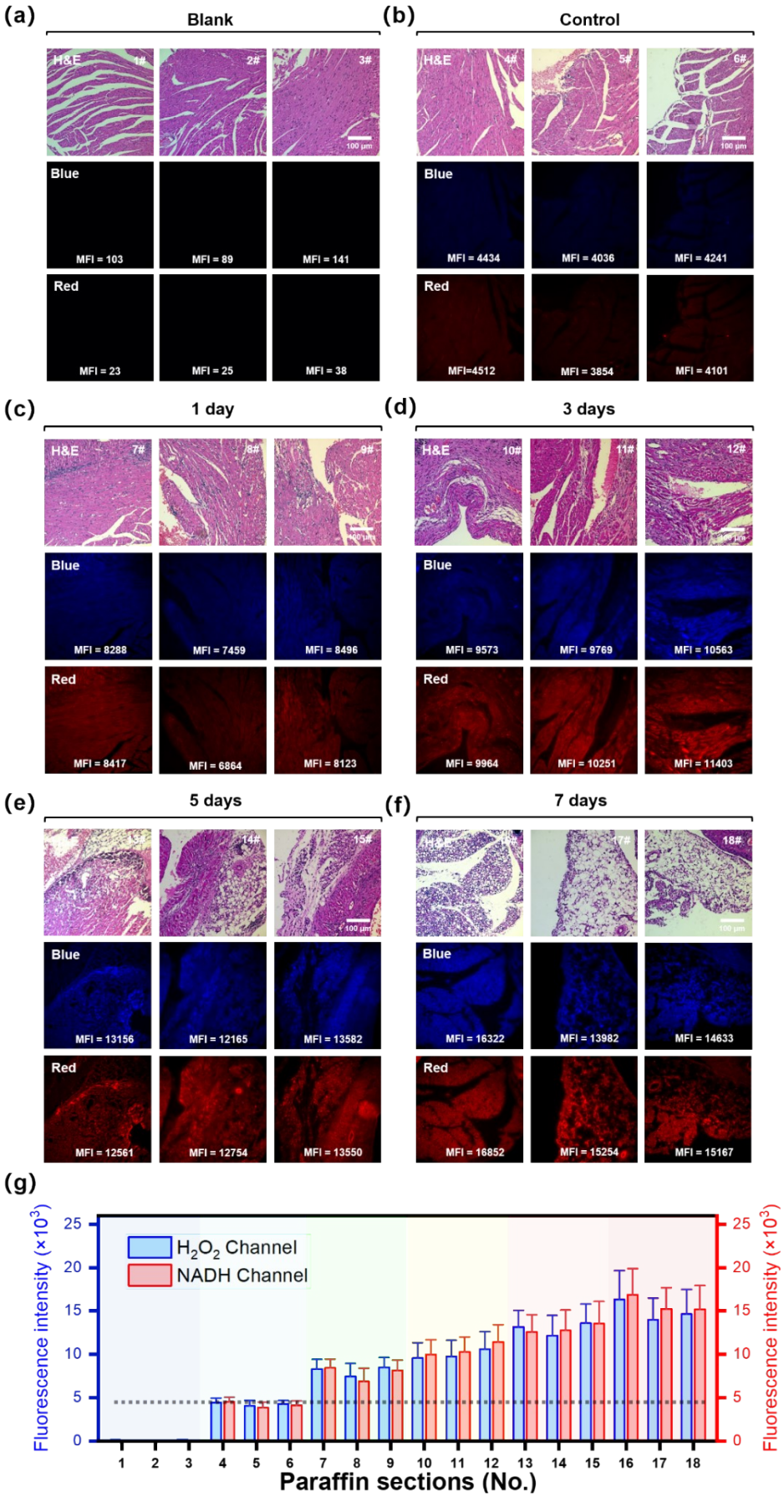


Figure S21. H&E and DQ1-stained histological analysis of infarcted and normal heart tissues. (a) Postoperative mice without injection of DQ1. (b) Normal group with DQ1 (1 mM, 200 μL) injection. (c) One day after myocardial infarction surgery with DQ1 (1 mM, 200 μL) injection. (d) Three days after myocardial infarction surgery with DQ1 (1 mM, 200 μL) injection. (e) Five days after myocardial infarction surgery with DQ1 (1 mM, 200 μL) injection. (f) Seven days after myocardial infarction surgery with DQ1 (1 mM, 200 μL) injection. (g) Mean fluorescence intensities of blue and red channel from each group. Mice were sacrificed and dissected after I.V. injection of DQ1 (1mM, 200 μL) for 12 h. Red channel, λ_{ex}: 540-580 nm λ_{em}: 600-660 nm, blue channel, λ_{ex}: 362-396 nm, λ_{em}: 432-480 nm. Scale bar: 100 μm.

2.15 H&E staining and fluorescence imaging of myocardial sections containing both ischemic and normal tissues

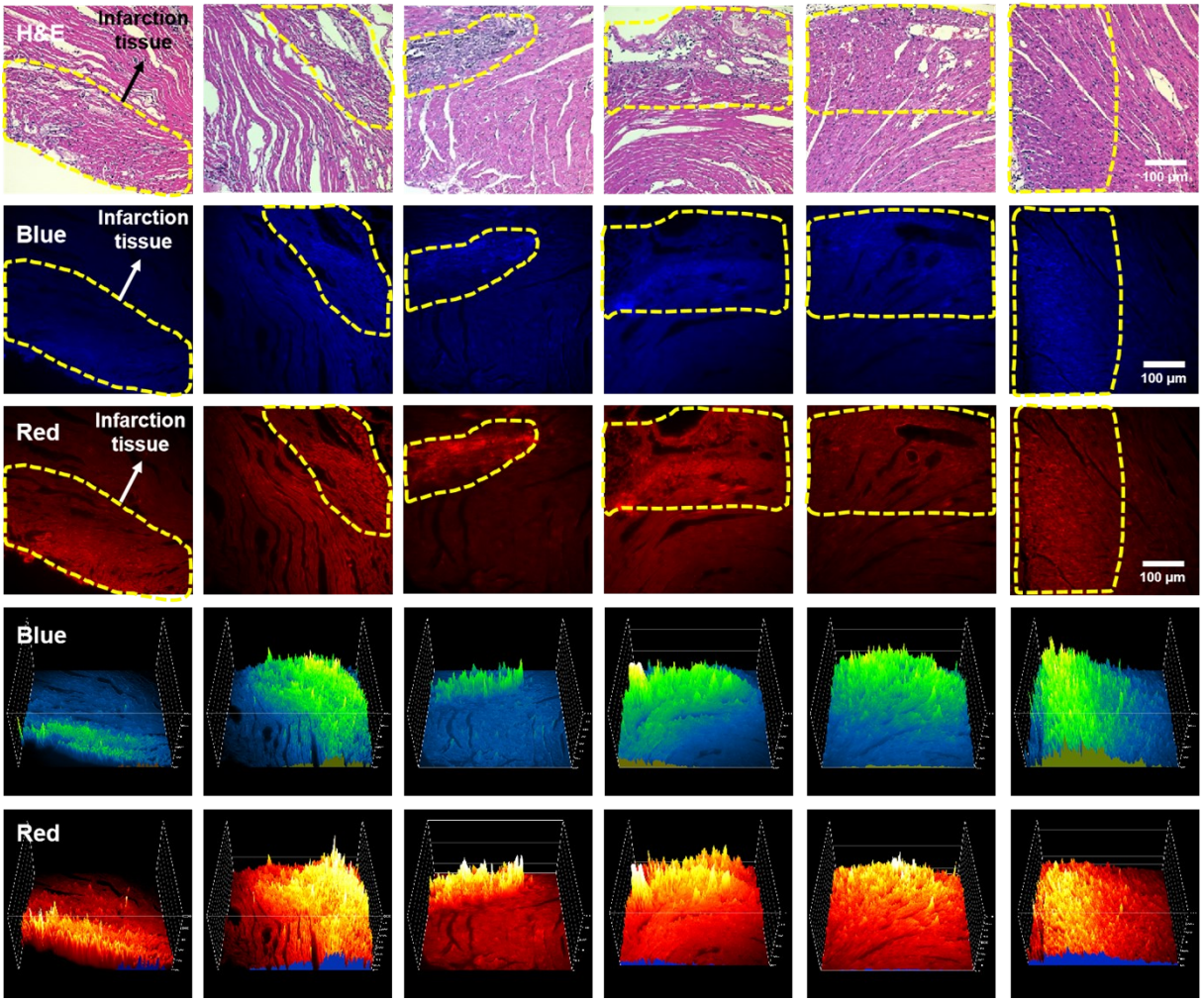


Figure S22. H&E staining, fluorescence imaging and 3D strength surface of myocardial sections containing both ischemic and normal tissues. Red channel, λ_{ex}: 540-580 nm λ_{em}: 600-660 nm, blue channel, λ_{ex}: 362-396 nm, λ_{em}: 432-480 nm. Scale bar: 100 μm.

2.16 Reagent kit assay of trichloroethylene sensitization

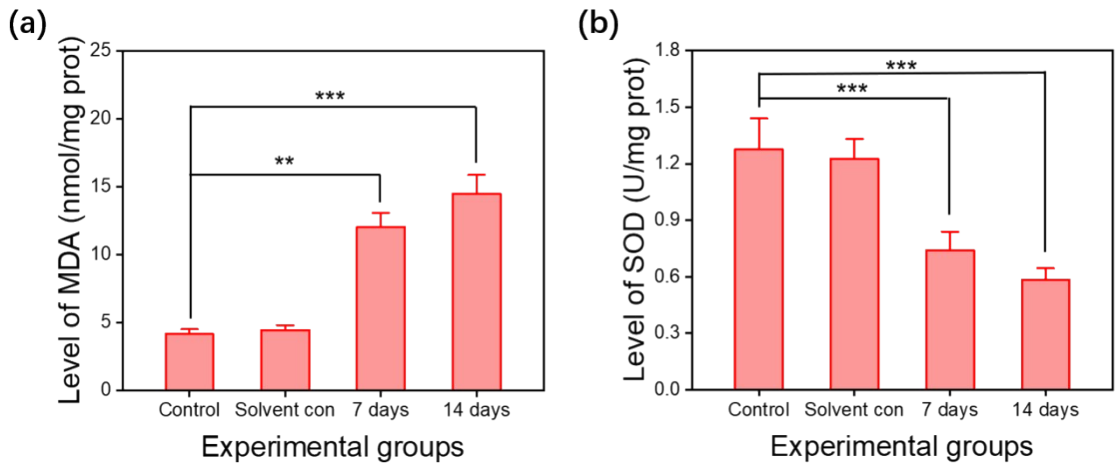


Figure S23. (a) Changes in liver homogenate MDA content subcutaneously injecting TCE. (b) Changes in liver homogenate SOD content after subcutaneously injecting TCE. (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$)

2.17 H&E and DQ1-stained histological analysis of liver tissues.

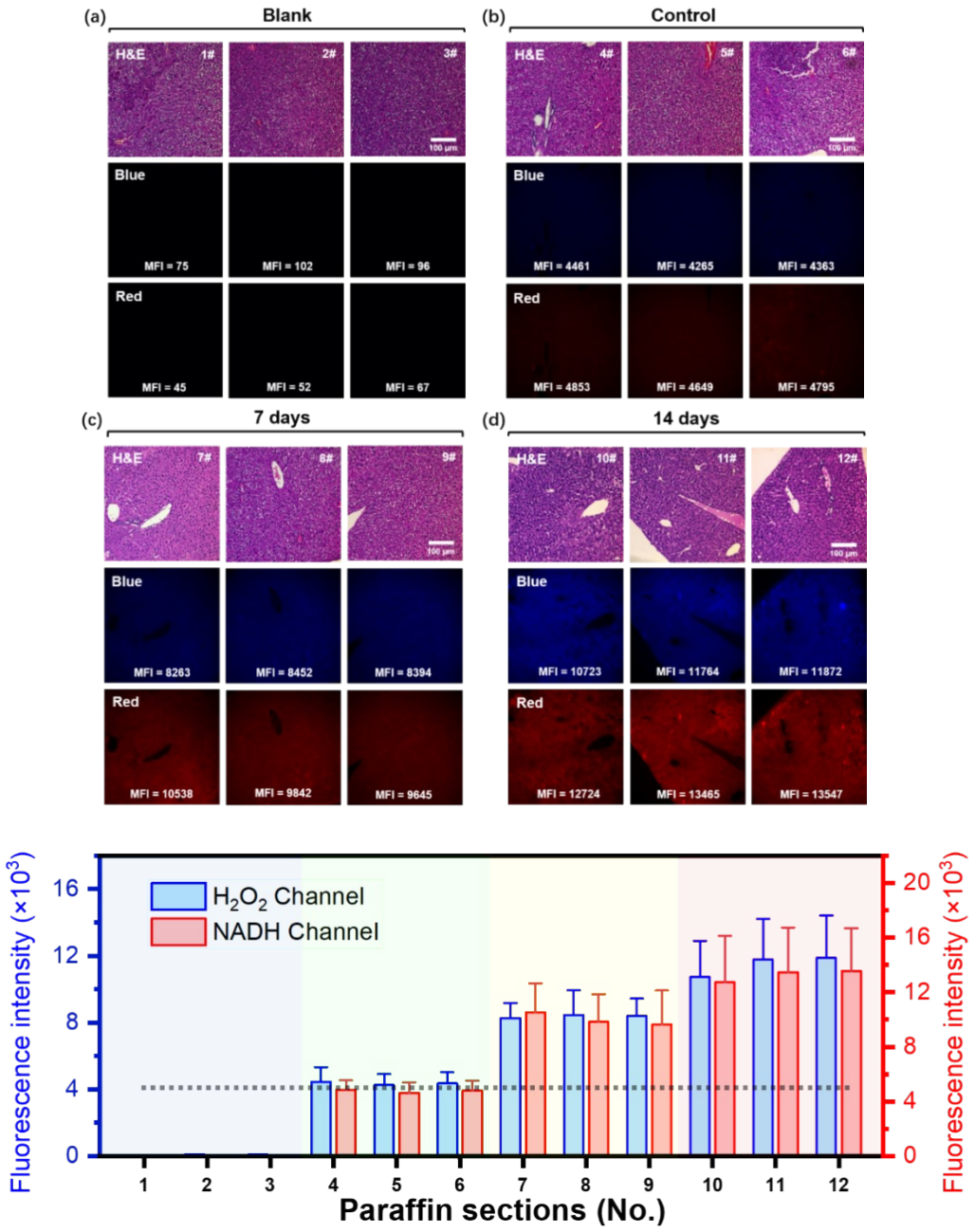


Figure S24. Images of H&E and DQ1 (5 μM) stained histological sections of liver tissues exposed to trichloroethylene (0, 7, 14 days). (a) Normal group without DQ1 injection. (b) Normal group with DQ1 (1 mM, 200 μL). (c) Seven days after subcutaneous TCE injection. (d) Fourteen days after subcutaneous TCE injection. (e) Mean fluorescence intensities of blue and red channel from each group. Mice were sacrificed and dissected after I.V. injection of DQ1 (1mM, 200 μL) for 12 h. Red channel, λ_{ex}: 540-580 nm λ_{em}: 600-660 nm, blue channel, λ_{ex}: 362-396 nm, λ_{em}: 432-480 nm. Scale bar: 100 μm.