Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

An extremely rapid-response fluorescence probe for hydrogen peroxide and its applications in living cells

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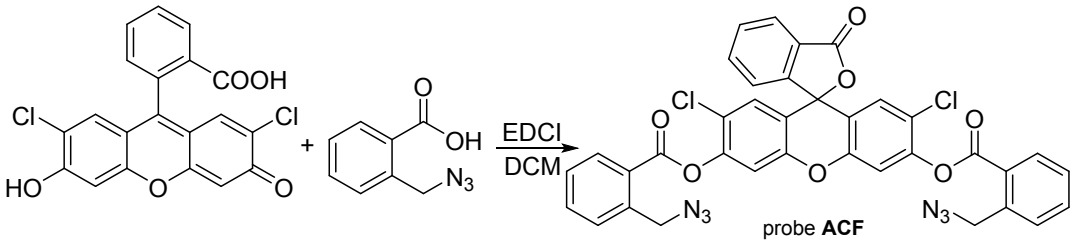
General information

All reagents and solvents were purchased from commercial suppliers and used without further purification unless otherwise stated. Deionized water was used throughout all experiments. All reactions were magnetically stirred and monitored by thin layer chromatography (TLC). Column chromatography was performed using 200-300 mesh silica gel supplied by Qingdao Marine Chemical Factor. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE AV400 at 400 and 100 MHz, respectively. All NMR chemical shifts were referenced to residual solvent peaks or to $Si(CH_3)_4$ as an internal standard, spectra recorded in $CDCl_3$ were referenced to residual $CHCl_3$ at 7.26 ppm for ^{1}H or 77.0 ppm for ^{13}C. All coupling constants *J* are quoted in Hz. FTIR spectra were obtained with a Bruker Vertex 70 FT-IR spectrometer with KBr pellets. All IR samples were prepared as thin film and reported in wave numbers (cm^{-1}). High resolution mass spectra were obtained on a Q-TOF6510 instrument mass spectrometer. Fluorescence spectra were carried out on an Edinburgh Instruments Ltd-FLS920 fluorescence spectrophotometer.

1. Synthesis and Characterization of Compounds

2',7'-dichlorodihydrofluorescein[1] and 2-(azidomethyl)benzoic acid[2] were prepared according to the reported methods previously.



A solution of 2-(azidomethyl)benzoic acid (529.4 mg, 3.0 mmol) in DCM (5 mL), was added EDCI (718.9 mg, 3.75 mmol) , DMAP (152.7 mg, 1.25 mmol) and 2',7'-dichlorodihydrofluorescein (500.0 mg, 1.25 mmol). The resulting mixture was warmed to room temperature and stirred for 12 h. The resulting solution was poured into water (30 mL) and extracted with DCM (3×20 mL). Combined organic layers were washed with brine, dried over anhydrous $MgSO_4$ and concentrated to

afford a red oil. Flash chromatography of the crude product (5:1 petroleum ether:ethyl acetate) provided the desired product as colorless oil (494.6 mg, 55%). Data for **ACF**: R_f 0.35 (petroleum ether:ethyl acetate = 5:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (dd, *J* = 7.9, 1.1 Hz, 2H), 8.10 (d, *J* = 7.5 Hz, 1H), 7.78 (td, *J* = 7.5, 1.2 Hz, 1H), 7.73 (dd, *J* = 7.5, 0.9 Hz, 1H), 7.69 (td, *J* = 7.6, 1.3 Hz, 2H), 7.61 (d, *J* = 7.2 Hz, 2H), 7.57 – 7.46 (m, 2H), 7.34 (s, 2H), 7.25 (d, *J* = 0.9 Hz, 1H), 6.96 (s, 2H), 4.88 (s, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.62, 163.59, 152.00, 149.82, 148.44, 138.79, 134.17, 132.03, 130.00, 129.07, 128.48, 126.48, 125.76, 125.58, 124.03, 122.85, 117.90, 113.03, 80.43, 52.98; Anal. Calcd for $C_{36}H_{20}Cl_2N_7O_7$: C, 60.09; H, 2.80; N, 11.68. Found: C, 59.74; H, 2.96; N, 10.94.

2. In *vitro* fluorescence spectroscopy measurement

General Procedure for H_2O_2 Detection

All UV−vis, fluorescence, and quantum yield measurements were carried out in 10 mM PBS buffer solution containing 5% CH_3OH, pH 7.4. In a 5 mL tube, PBS buffer (4 mL) and 250 μL **ACF** (100 μM in CH_3OH) were mixed, and then 4 mM H_2O_2 solution (50 μL, 80 eq) was added. The final solution volume was adjusted to 5 mL with PBS buffer to obtain a final concentration of 5 μM. After rapid mixing of the solution, it was transferred to a 10 × 10 mm quartz cell and incubated at 37 °C for in vitro detection. Fluorescence spectra were recorded in the range from 480 to 700 nm with λ_{ex} = 450 nm, and absolute emission quantum yields were determined accordingly.

Quantum Yields

Fluorescence quantum yields of **ACF** was determined in PBS buffer (10 mM, pH 7.4) with fluorescine (Φ = 0.65, in water) as a reference. 2',7'-dichlorodihydrofluorescein was obtained in the experiment by addition of 80 eq of H_2O_2 to the solution of probe **ACF**. The quantum yields were calculated using an Eq follows:

$$\Phi_u = [(A_s F A_u \eta^2)/ (A_u F A_s \eta_0^2)]\Phi_s.$$

Where A_s and A_u are the absorbance of the reference and sample solution at the reference excitation wavelength, FA_s and FA_u are the corresponding integrated fluorescence intensity, and η and η_0 are the solvent refractive indexes of sample and reference, respectively. Absorbance of sample and reference at their respective excitation wavelengths was controlled to be lower than 0.05.

Quantum yield of **ACF**: Φ = 0.0024

Quantum yield of **ACF**+H_2O_2: Φ = 0. 6780

Detection limit

To determine the detection limit, the emission intensity of probe **ACF** without H_2O_2 was measured by 10 times and the standard deviation of blank measurements was determined. The detection limit was then calculated with the equation: detection limit = 3σ/k, where σ was the standard deviation of blank measurements, k was the slope between intensity difference versus sample concentration. According to fluorometric method, the detection limit of probe **ACF** for H_2O_2 was determined as 6.5 nM.

Detection limit of **ACF** =3σ/slope=3*0.008791473/4.05921=0.0065 μM

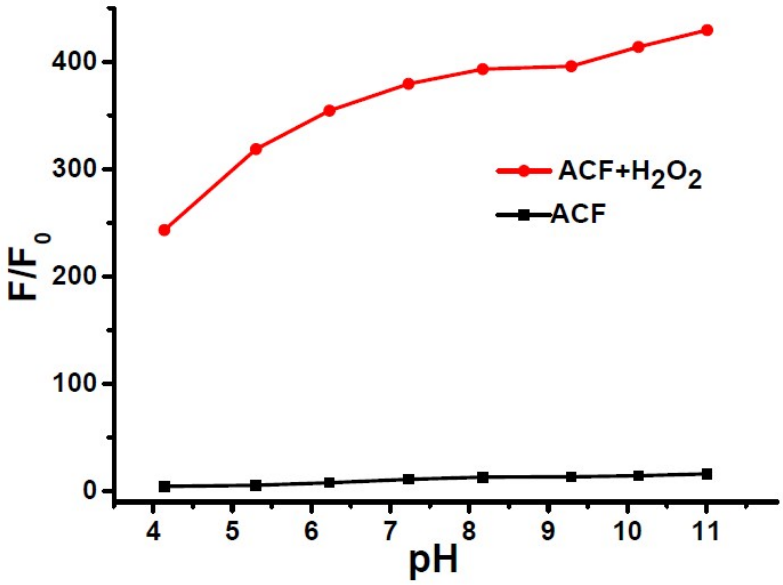


Fig.S1 Fluorescence response of **ACF** (5 μM) and **ACF** (5 μM) incubated with 80 eq of H_2O_2 in CH_3OH/PBS buffer (10 mM, 5:95, pH from 4 to 11) for 90 min. λ_{ex} = 450 nm, λ_{em} = 527 nm. Slits: 5/5 nm.

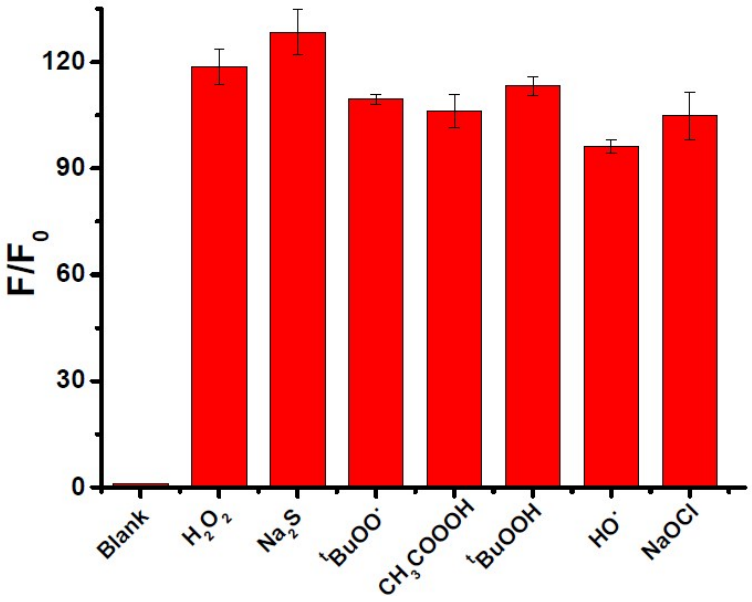


Fig.S2 Fluorescence responses of **ACF** (5 μM) to H_2O_2 and other ROS and H_2S (80 eq) in CH_3OH/PBS buffer (10 mM, pH = 7.4, 5/95) for 5 min. 1. Blank; 2. H_2O_2; 3. Na_2S; 4. tBuOO·; 5. CH_3CO_3H; 6. *t*BuOOH; 7. HO·; 8. NaOCl. λ_{ex} = 450 nm, λ_{em} = 527 nm. Slits: 5/5 nm.

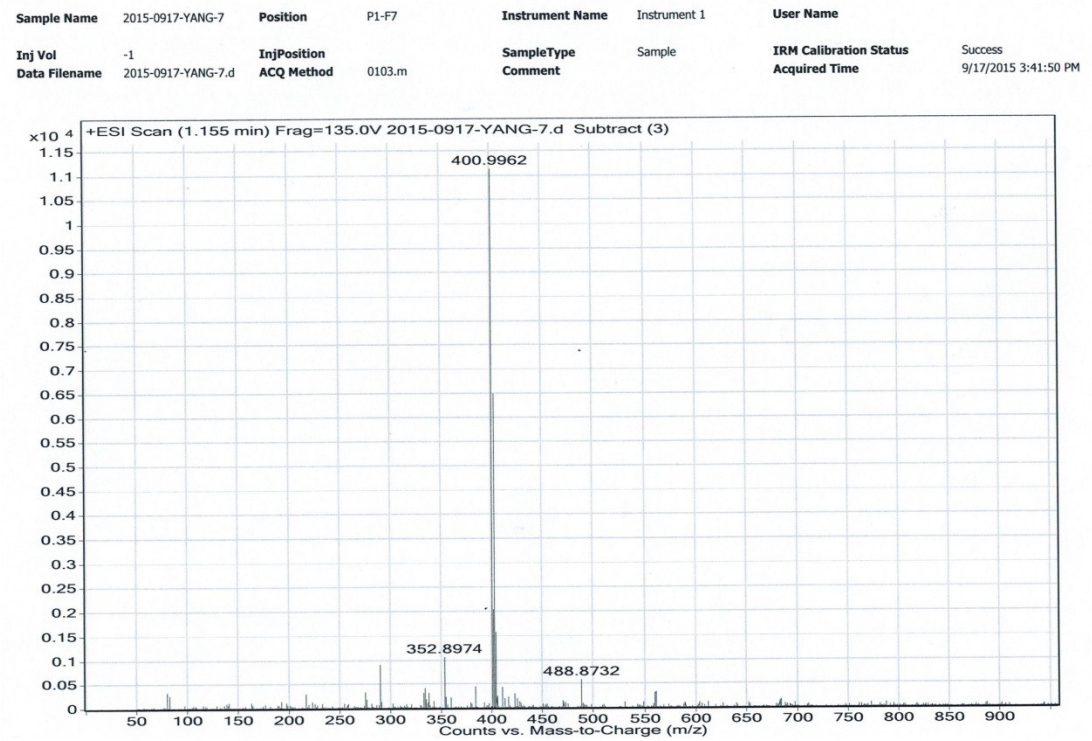


Fig.S3 HRMS chart of **ACF** treated with H_2O_2 (80 eq).

3. MTT assay

The MTT assay was used to evaluate the cytotoxicity of probe. A-549 cells and Raw 264.7 cells were cultured in DMEM medium supplemented with 10% fetal

bovine serum at 37 °C in an atmosphere containing 5% CO_2. A-549 cells and Raw 264.7 cells were seeded onto 96-well plates at a density of 1×104 cells/well and incubated for 24 h. The medium was replaced by various probe over a range of concentrations (0.5 μM to 30 μM) dissolved in culture medium. After incubation at 37 °C, 5% CO_2 for 24 h, each well of cells were treated with 20 μl MTT solution (5 mg/mL), and incubated for another 4 h. After that, the medium was removed, and 100 μL of DMSO were added to dissolve the formazan crystals. The plate was agitated for 10 min, and each well was finally analyzed by the microplate reader (Thermo Scientific, Multiskan FC) and detected by the absorbance at 570 nm.

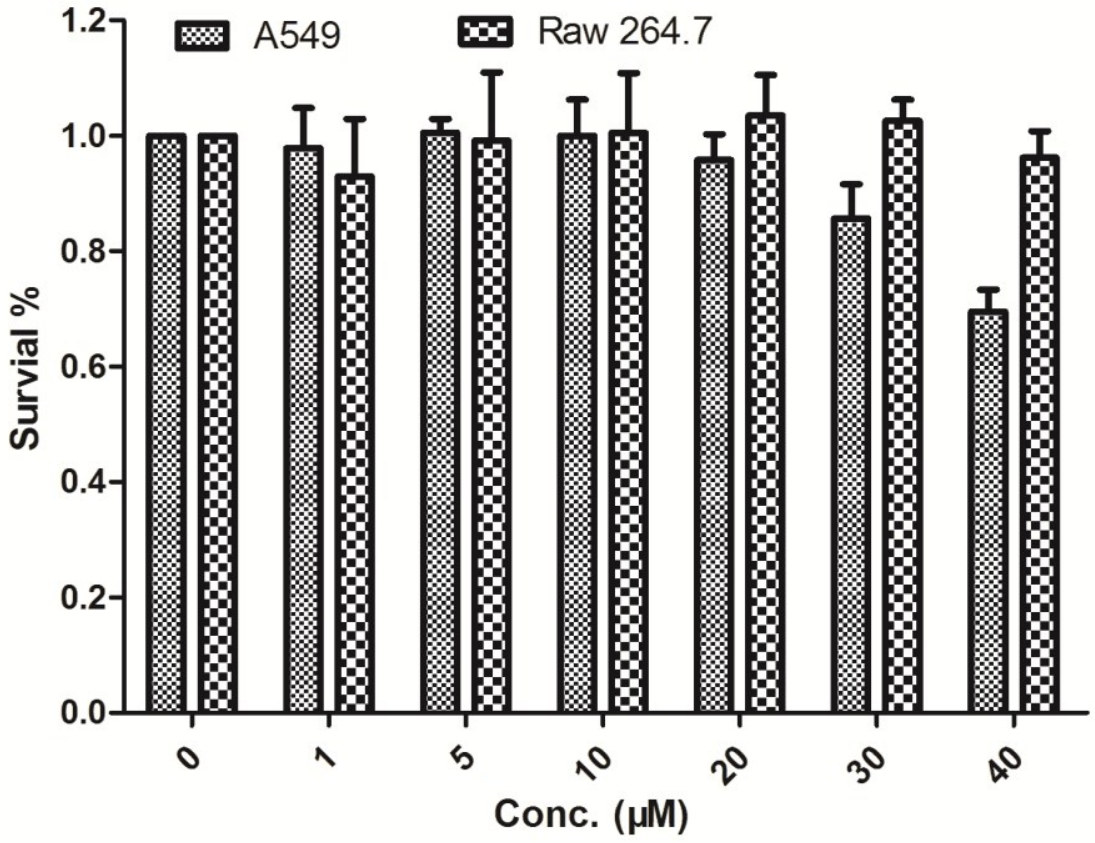


Fig.S4 The cytotoxicity of the probe **ACF** evaluated by the MTT assay

4. Cell culture and fluorescence imaging

The HeLa cells were cultured in DMEM medium supplemented with 10% fetal bovine serum at 37 °C in an atmosphere containing 5% CO_2. For live cells imaging, probe **ACF** was added to the cells and incubated for 10 min and washed with PBS (phosphate-buffered saline) three times. After replacement of the medium, cells

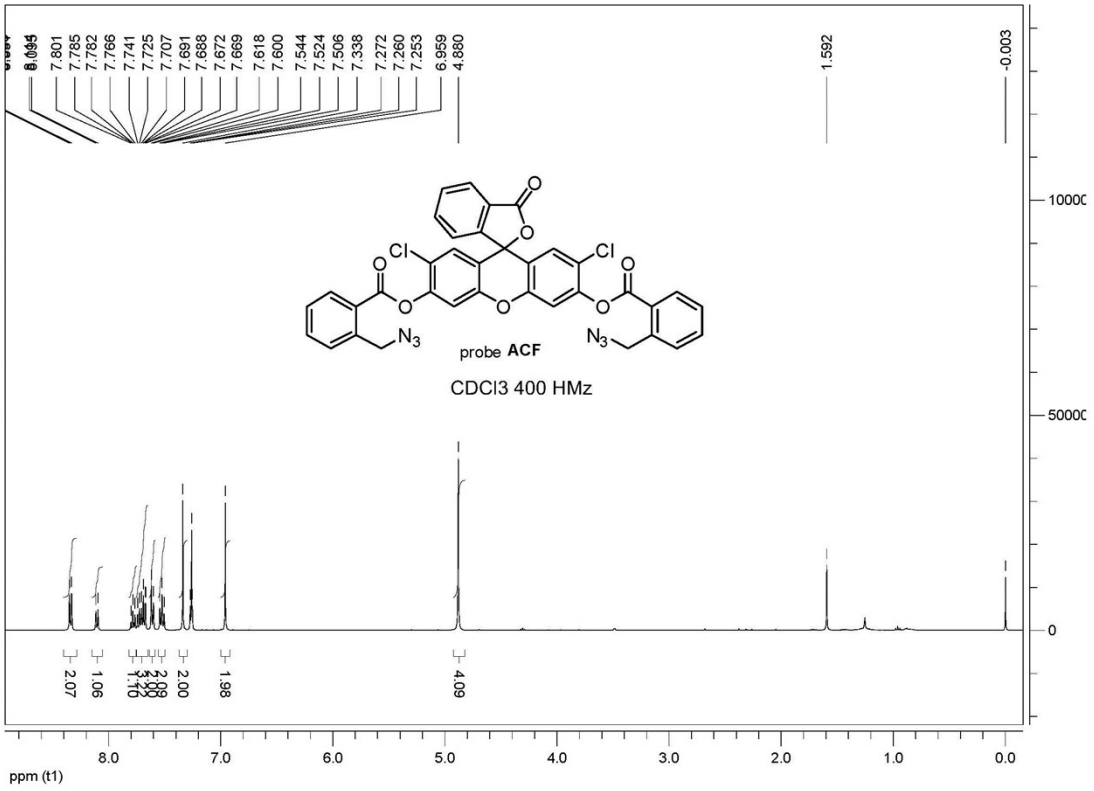
were imaged using an Olympus (FV1000) confocal laser scanning microscope with a CCD camera.

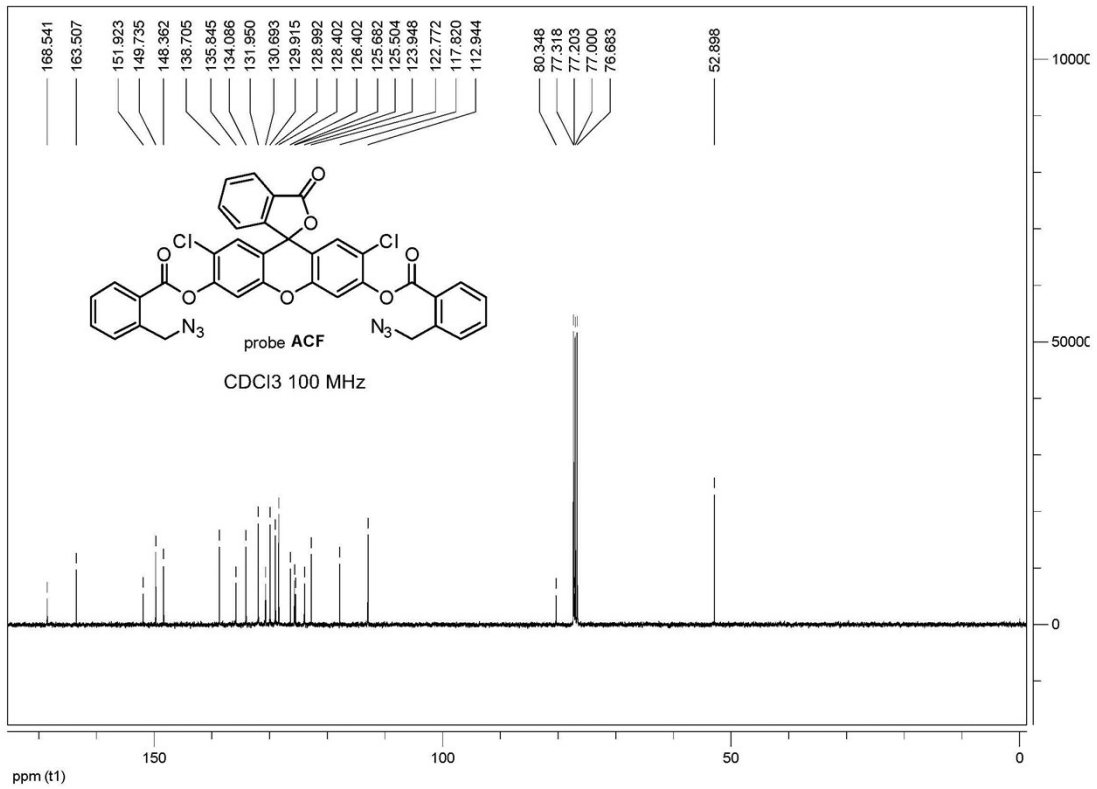
5. R references

1. M. G. Choi, J. O. Moon, J. Bae, J. W. Lee and S.-K. Chang, Org. Biomol. Chem., 2013, **11**, 2961.

2. Z. Wu, Z. Li, L. Yang, J. Han and S. Han, Chem. Commun., 2012, **48**, 10120.

7. ^{1}H NMR, ^{13}C NMR chart of compounds ACF

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