Supporting Information

Double-mode detection of HClO by naked eye and concurrent fluorescence increasing in absolute PBS

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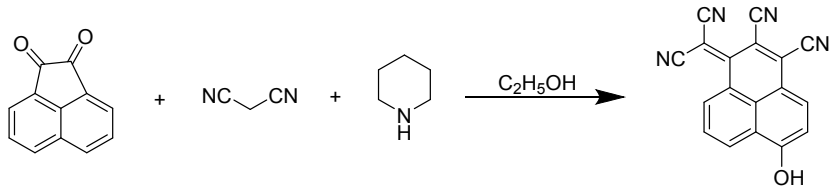
Materials and apparatus

All solvents and chemical reagents were analytical grade and purchased from commercial suppliers. ^{1}H NMR spectra were recorded on a Bruker 500 MHz AVANCE III spectrometer with chemical shifts reported in ppm at room temperature. Mass spectra were obtained with Thermo Fisher LCQ Fleet mass spectrometer (USA) and a LC/Q-Tof MS spectrometry (USA). Absorption spectra were collected by using a Shimadzu 1750 UV-visible spectrometer (Japan). Fluorescence spectra were measured with a Shimadzu RF-5301 fluorescence spectrometer (Japan). The pH of the testing systems was determined by a PHS-3C pH Meter (China). Cell toxicity test of WCN was tested by microplate reader (KHB ST-360). The confocal laser microscope data were acquired using a confocal fluorescence microscope (OLYMPUS FV-1000). The mouse imaging was conducted by a vivo imaging system FX Pro (Kodak In-Vivo imaging system FX Pro, USA).

All of the experiments were performed in compliance with the relevant laws and institutional guidelines, and were approved by Northwest A&F University.

Synthetic procedures of probe WCN

Acenaphthene-1,2-dione (1.82 g, 1 equiv, 10 mmol) and malononitrile (1.32 g, 2equiv, 20 mmol) were dissolved in absolute ethyl alcohol (60 mL). The solution was refluxed for 3 h under strong magnetic stirring. Note that the yellow mixture slowly took into a deep green color upon heating. After cooling at 0 °C, filtration of the reaction mixture yielded a green dark powder. Purification of the crude over silica gel column chromatography afforded a dark blue powder. The polarity of the starting eluent (dichloromethane / ethyl acetate = 30:70) was slowly increased up to 100% ethyl acetate. ^{1}H NMR (500 MHz, DMSO): δ 9.19 (d, J = 8.0 Hz, ^{1}H), 8.49 (d, J = 7.5 Hz, ^{1}H), 7.74 (t, J = 7.9 Hz, ^{1}H), 7.59 (d, J = 9.1 Hz, ^{1}H), 7.10 (d, J = 9.1 Hz, ^{1}H). ESI-MS ($C_{18}H_6N_4O$), $[M-H]^-$, calc. m/z =293.05, found m/z = 293.17.



Water solubility of WCN

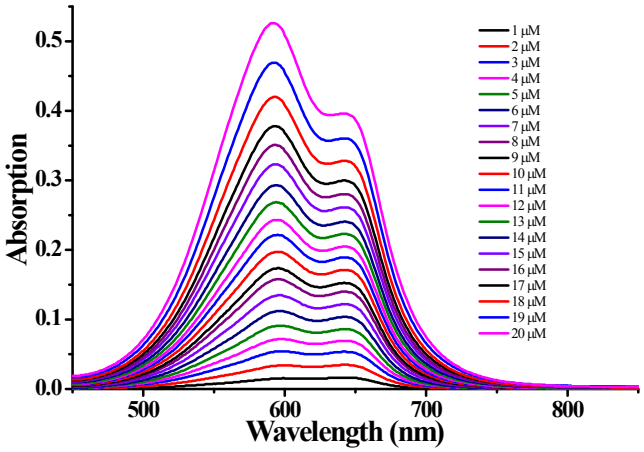


Fig. S1 The absorbance of various concentrations of **WCN** in PBS (20 mM, pH=7.4).

Absorption properties of WCN

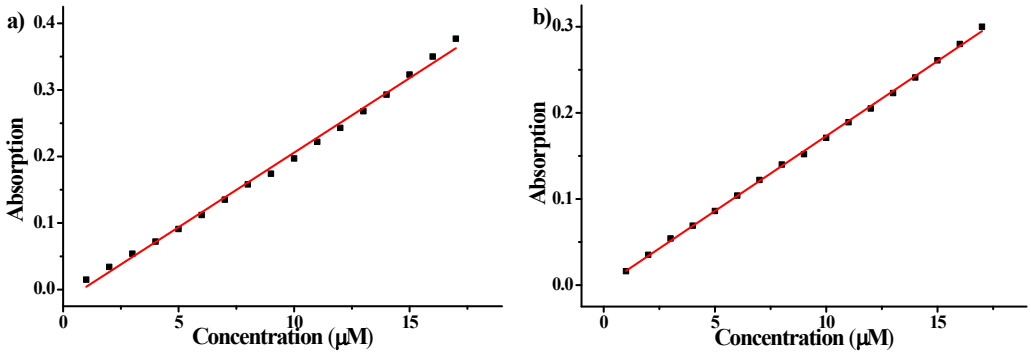


Fig. S2 (a) The linear relationship between the absorption at 595 nm and **WCN** concentrations. (b) The linear relationship between the absorption at 645 nm and **WCN** concentrations.

Dynamics of WCN to HClO

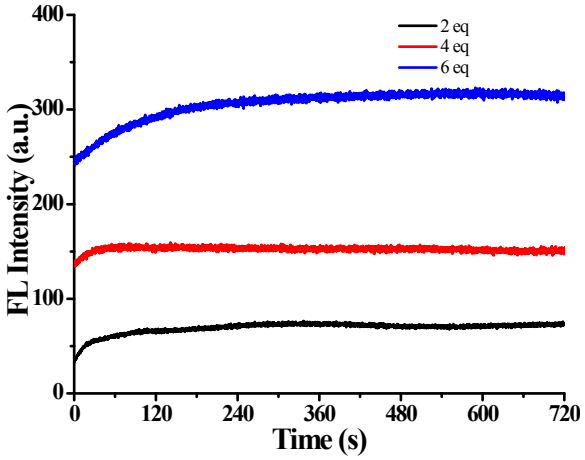


Fig. S3 Time-dependent fluorescence intensity changes of **WCN** (5 μM) at 560 nm upon addition of varied concentrations of HClO. All data were recorded in PBS buffer (20 mM, pH 7.4) at room temperature. λ_{ex} = 530 nm.

Absorption response to HClO

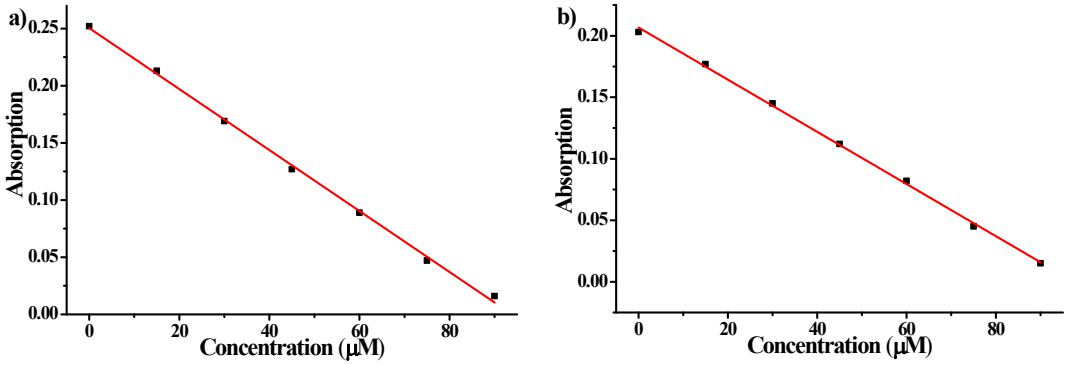


Fig. S4 (a) The linear relationship between the absorption of **WCN** at 595 nm and HClO concentrations. (b) The linear relationship between the absorption of **WCN** at 645 nm and HClO concentrations.

Fluorescence response to HClO and other analytes

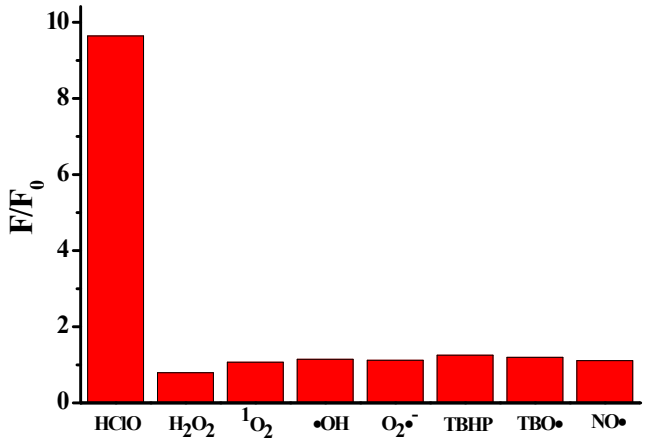


Fig. S5 Fluorescence ratio (F/F_0) at 560 nm of **WCN** (5 μM) with HClO (10 μM) in the presence of various ROS/RNS (100 μM) in PBS buffer (20 mM, pH 7.4) at room temperature. λ_{ex} = 530 nm.

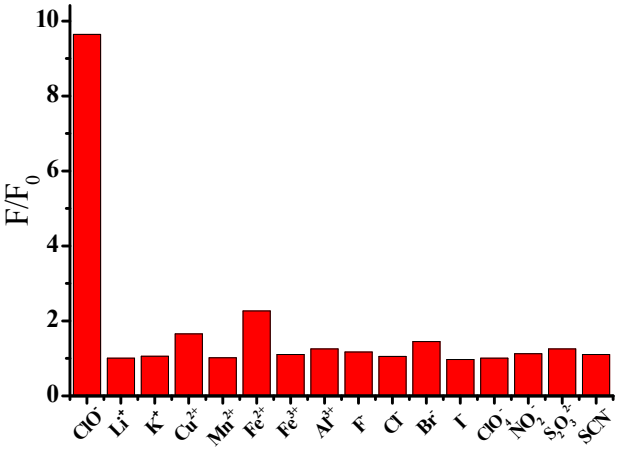


Fig. S6 Fluorescence spectra of **WCN** (5 μM) upon the addition of ClO^- (10 μM) and other analytes (100 μM for each) respectively in PBS buffer (20 mM, pH 7.4) at room temperature. All data were collected 5 min after the addition of the above analytes. λ_{ex} = 530 nm, λ_{em} = 560 nm.

The effect of pH for WCN

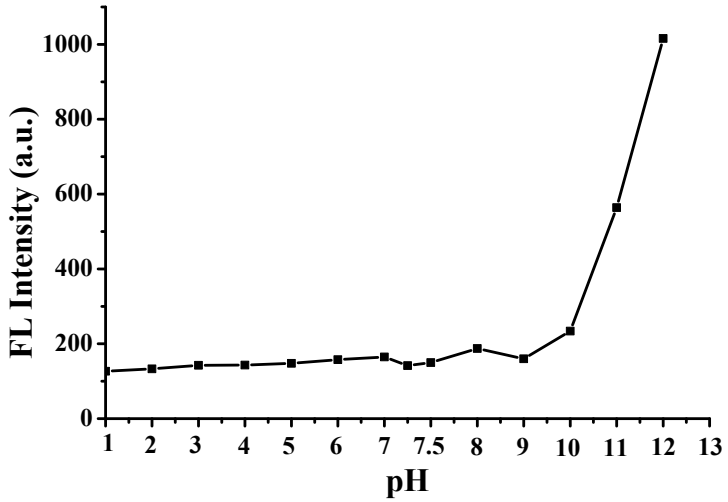


Fig. S7 The effect of pH value on the fluorescence intensity of **WCN** (10 μM).

The effect of solution on probe WCN

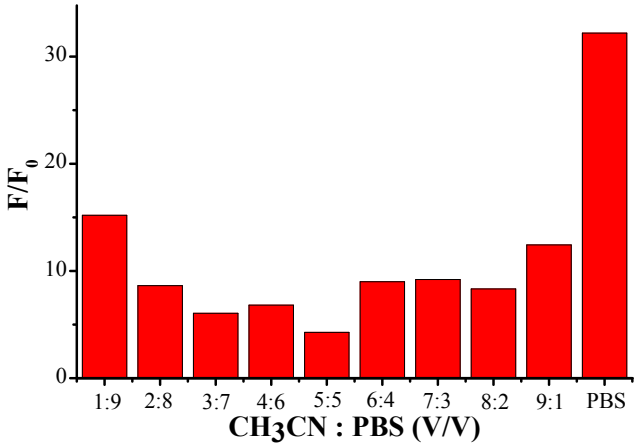


Fig. S8 Fluoresence intensity of **WCN** (5 μM) in different ratio of PBS (20 mM, pH = 7.4) and CH_3CN with the addition of HClO (25 μM) at room temperature. λ_{ex} = 530 nm, λ_{em} = 560 nm.

Cell toxicity test of WCN

Briefly, Hela cells were seeded in 96-well microplates at a density of 1×10^5 cells/mL in 100 μL medium containing 10% FBS. After 24 h of cell attachment, plates were washed with 100 uL/well phosphate buffered saline (PBS) and then cells were cultured in medium with various concentrations (2.5-20 uM) of **WCN** for 24 h. 10 uL of MTT (5 mg/mL) prepared in PBS was added to each well and the plates were incubated at 37 °C for 4 h in a 5% CO_2 humidified incubator. The medium was then carefully removed, and the purple products were lysed in 200 uL dimethyl sulfoxide (DMSO). The plate was shaken for 10 min and the absorbance was measured at 492 nm using a microplate reader (KHB ST-360).

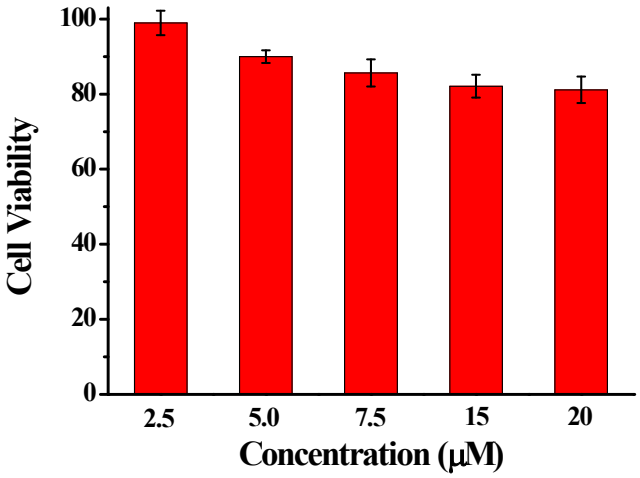


Fig. S9 Cell viability of Hela cells in the presence of different concentrations of **WCN** after 24 h of incubation determined by the MTT assay. Error bars standed for the mean value of five experiments.

Comparison about the detection limits for HClO

Table S1 Comparison table about the detection limits for HClO

Probe	Linear range	Solvent	LOD (M)
Ref. 6f	0−10 nM	PBS-EtOH, 9:1	5.6×10^{-10}
Ref. 6g	0–80 μM	PBS-0.05% DMSO	1.79×10^{-8}
Ref. 7e	2–20 μM	PBS	3×10^{-7}
Ref. 7f	0–0.5 μM	PBS-CH_3CN, 1:9	9.3×10^{-8}
Ref. 7g	0–100 μM	CH_3CN-Water, 4:1	2.8×10^{-8}
Ref. 7h	0–10 μM	PBS-0.5% DMSO	4.3×10^{-7}
This work	0–60 μM	PBS	7.7×10^{-11}

Characterization

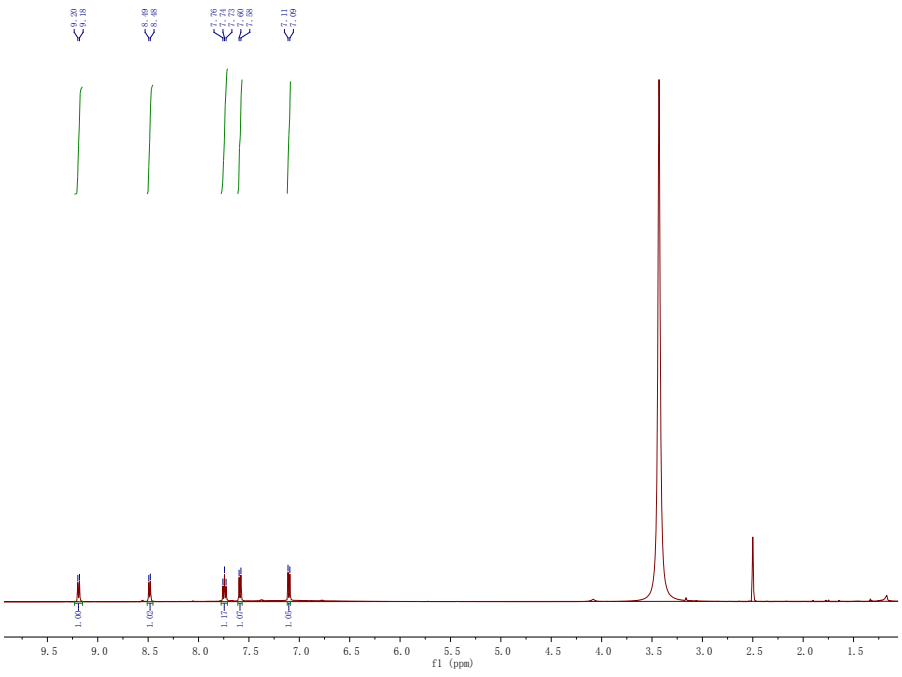


Fig. S10 ^{1}H NMR of **WCN** in DMSO-d_6.

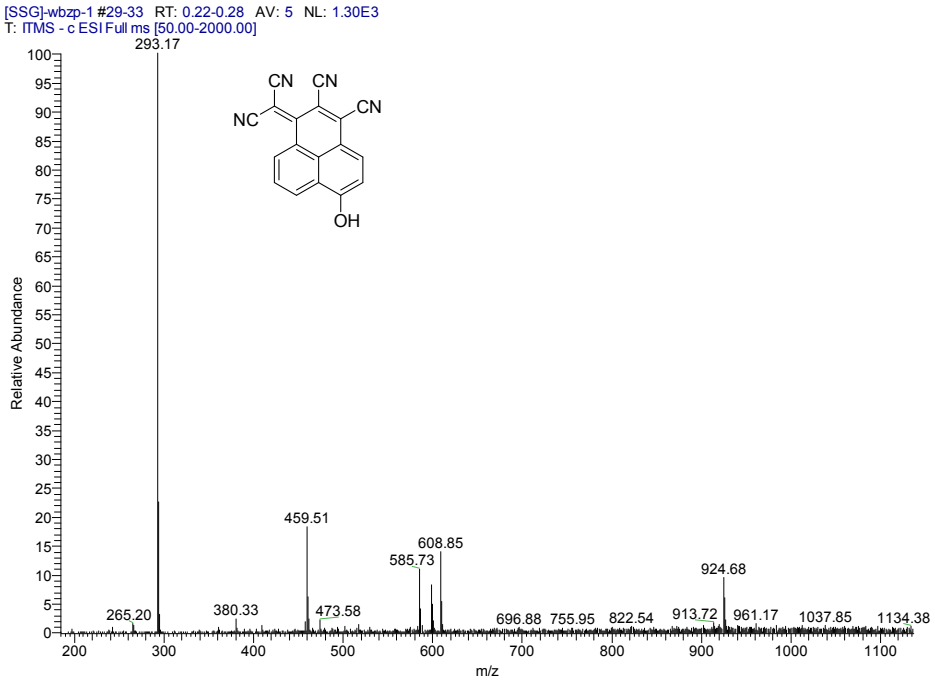


Fig. S11 ESI-MS of **WCN**

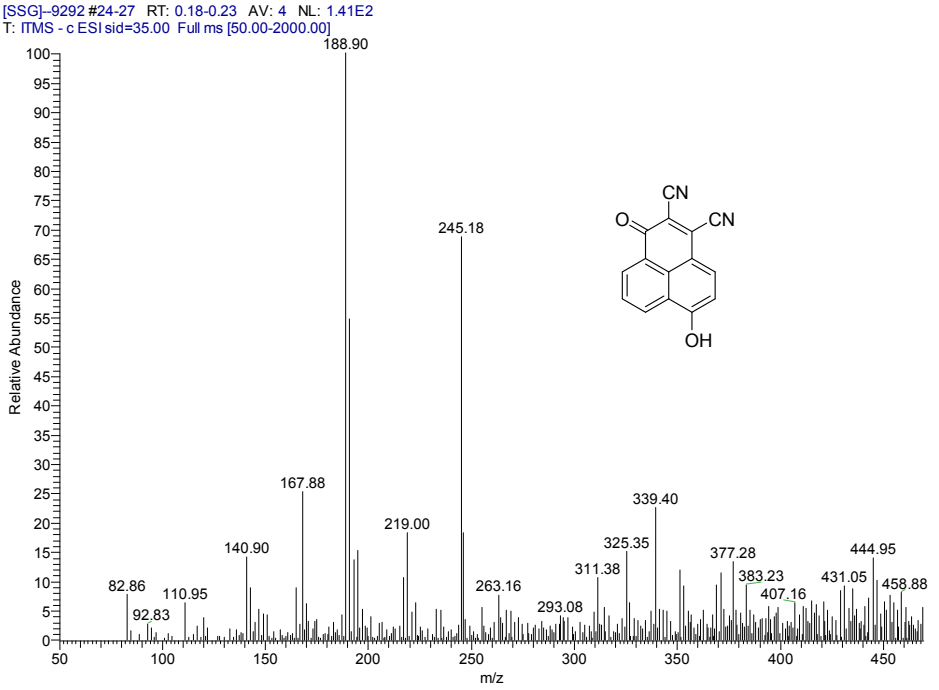


Fig. S12 ESI-MS spectra of **WCN** + HClO.