Supporting Information for

A fluorescent probe for the detection of N_2H_4 in solution, steam, and biological system

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Materials and instruments

All reagents and materials were purchased from commercial companies and used without further purification unless otherwise stated. Twice Distilled water was used in all experiments. 2'-hydroxyacetophenone, 4-hydroxybenzaldehyde, acetyl chloride，methanol (MeOH), sodium hydroxide (NaOH), H_2O_2, hydrochloric acid (HCl), dichloromethane (DCM), triethylamine (TPA), Dimethyl sulfoxide (DMSO), Phosphate Buffered Saline (PBS), Zn^{2+}, Mn^{2+}, Cu^{2+}, Mg^{2+}, K^{+}, Na^{+}, Ca^{2+}, Co^{2+}, Hg^{+}, F^{-}, Br^{-}, OAc^{-}, HSO_3^{-}, $S_2O_3^{2-}$, SO_3^{2-}, SO_4^{2-}, NO_2^{-}, SCN^{-}, PO_4^{3-}, a-D-Glu (a-D-Glucose), cellulase, lysozyme, pepsase, Ala (Alanine), His (Histidine), Hcy, Asp (Asparagicacid), GSH (Glutathione), Ser (Serine) and N_2H_4.

All aqueous solutions were prepared with ultra-pure water obtained from a Milli-Q water purification system (18.2 MΩ cm).

^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker Avance III HD 500 MHz NMR spectrometer (Germany). High resolution mass spectrometric (HRMS) analyses were measured on Aglient 6550 Q-TOF. The absorbance was recorded by ultraviolet-visible absorption spectrometry (UV-2700, Shimadzu) or microplate reader (TransGen Biotechnology). TLC analysis were carried out on silica gel plates and column chromatography was conducted over silica gel (mesh 200-300), both were purchased from the Qingdao Ocean Chemicals. Cells were photographed under the microscope (RVL-100-G, USA Discover-Echo, 60×).

Preparation of Solutions of probe HFOAc and Analytes

Unless otherwise stated, all tests are carried out according to the following procedures. A probe solution of **HFOAc** (1.0 mM) was prepared in DMSO. After the final volume was adjusted to 10 mL with PBS buffer (50% DMSO), placed at 37℃ for 20 minutes. Then 2 mL portion was transferred to a 1 cm quartz cell to measure absorbance and fluorescence. All fluorescence measurements were made on Hitachi F4600 Fluorescence Spectrophotometer. By adding the minimum volume of sodium NaOH (0.1 M) or HCl (0.2 M), the pH of the solution changed slightly.

Determination of the detection limit

The detection limit was determined using the fluorescence titration. To get the slope, the fluorescence intensity (at 530 nm) was plotted as the N_2H_4 concentration increased. The detection limit was obtained using the equation:

$$\text{Detection Limit} = 3\sigma/k$$

Where σ is the standard deviation of blank measurement (σ = 22.34483), k is the slope between the fluorescence intensity versus the concentrations of N_2H_4 (k = 401.36286), so the detection limit is 0.164 μM.

Paper strip application

Solution application

The cellulose filter paper was immersed in **HFOAc** solution with 100 μM DMSO for 1 min and dried in a vacuum drying oven (25 °C). Spray each paper with deionized water (0 %, 1 %, 10 %) N_2H_4 solution at room temperature. Fluorescence changes were observed with a camera under UV light (365 nm).

Vapor application

The cellulose filter paper was soaked in DMSO solution of **HFOAc** (100 μM) and dried in a vacuum drying oven (25 °C). Each paper was attached to the cap of a 20 mL small bottle and steamed with N_2H_4 (0 %, 0.5 %, 1 %, 5 %, 10 %) for 1min. Under ultraviolet (365 nm) irradiation, the fluorescence changes were monitored with a camera.

Culture and preparation of HeLa cells

HeLa cells were cultured in DMEM (Dulbecco's modified Eagle's medium) with 10% fetal bovine serum (FBS) in 5% CO_2 and 95% air at 37 ℃. Before the experiment, HeLa cells were inoculated in a 35 mm glass-bottomed culture dish with a density of 2×10^5 cells per dish, and then cultured in 2 mL culture medium at 37 ℃ in an incubator containing 5% CO_2 and 95% air for 24 hours. During this period, cells

will adhere to the glass surface. In use, HeLa cells were treated with **HFOAc** at 37℃ for 5 minutes.

Cytotoxicity assays

Cells were inoculated into 96-well plates, and probe **HFOAc** (95% DMEM and 5% DMSO) of 0, 1, 2, 5, 10, 30, 40, and 50 μM (final concentration) were added respectively. Subsequently, the cells were cultured at 37 °C in an atmosphere of CO_2 (5%) and air (95%) for 24 hours. Then cells were washed with PBS buffer and DMEM medium was added. Next, MTT (10 μL, 5 mg/mL) was injected into each well and incubated for 4 hours. Treatment with sodium dodecyl sulfate solution (100 μL) in H_2O - DMF mixture produced purple methyl. The viability of cells was determined by assuming that the viability of cells without **HFOAC** was 100%.

Preparation of cell imaging experiment.

The HeLa cells were plated at 1×10^5 cells / mL suspension in μ-slide 8 well and allowed to culture overnight, respectively. Then the cells were treated with N_2H_4 (50 μM, 100 μM, 200 μM) for 20 min with fresh culture medium. HeLa cells untreated with N_2H_4 were used as control. 10 μM of **HFOAC** was added into the cell groups for 5 min. The cell images were obtained using the microscope (RVL-100-G, USA Discover-Echo, 60×).

Statistical Analysis

The data were expressed as mean ± SD. The error bar represents the standard deviation, n = 3. Images were quantified by fluorescence analysis using ImageJ.

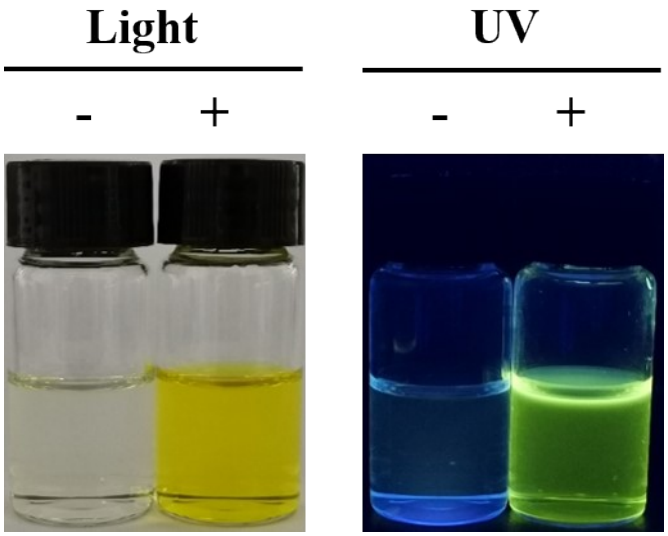

Fig. S1. Color changes of HFOAc without N_2H_4 treatment (left) and with N_2H_4 treatment (right) under 365 nm UV and natural light excitation respectively.

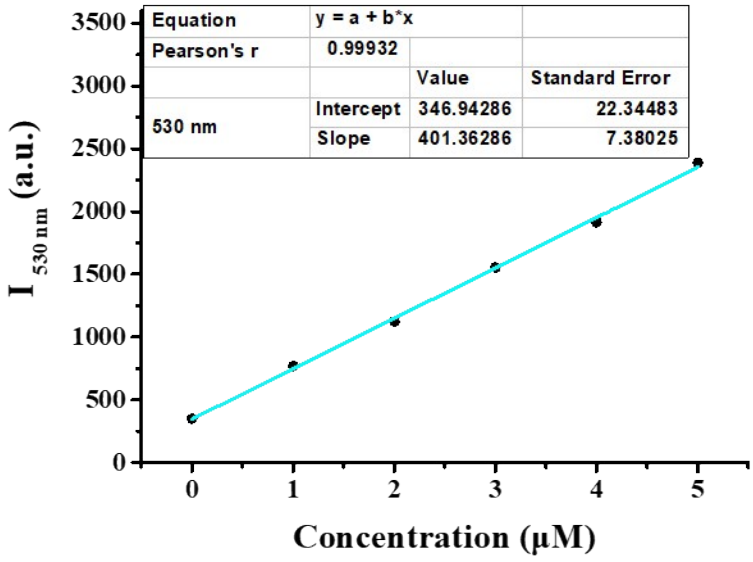

Fig. S2. The linear function for the fluorescence intensity of HFOAc with the concentration of N_2H_4 at 530 nm.

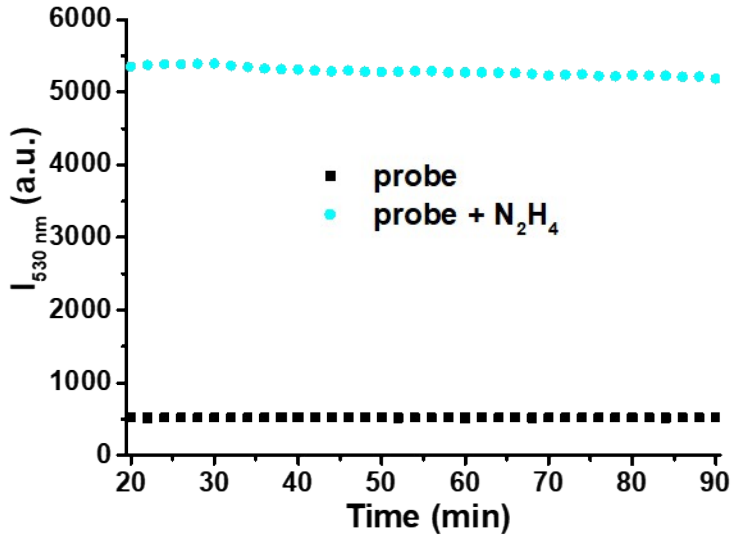

Fig. S3. The photostability of the probe HFOAc before and after the addition of N_2H_4 (500 μM).

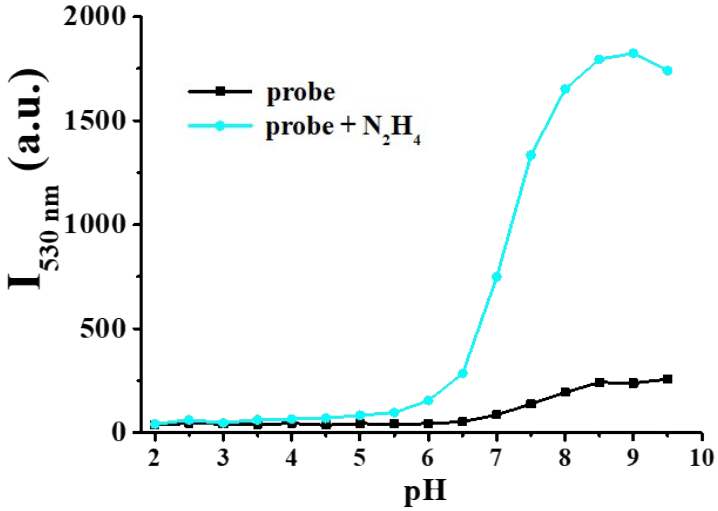

Fig. S4. The fluorescence changes of HFOAc at different pH values. Reaction time: 30 min.

Table S1. Comparison of the properties.

Probe Name	Synthesis	Detection limit	Response times	Fluorescence strategy	Source
HFOAc	2 steps	0.164 μM	4 min	ICT-ESIPT Turn-on	This work
RhodCl-Hz	2 steps	0.64 μM	50 min	ICT-ESIPT Turn-on	1
CDs-MnO_2	/	0.15 μM （4.8 ppb）	2 min	/	2
Cy-OAc	4 steps	0.48 ppb	60 min	PET Turn-off	3
TPE-PMI	2 steps	6.4 ppb	/	ICT-ESIPT Turn-on	4
Cou-Lyso-N_2H_4	2 steps	3.93 μM	60 min	ICT-ESIPT Turn-on	5
BRBA	2 steps	0.1 μM	35 min	ICT-ESIPT Turn-on	6
1	2 steps	2.90 ppb	45 min	AIE-ICT Turn-on	7
Probe	/	1.45 ppb （0.045 μM）	60 min	PET-ESIPT Turn-on	8
CyOE	5 steps	2.58 ppb	15 min	ICT-ESIPT Turn-on	9
Cou-1-N_2H_4	2 steps	11.9 nM or 0.38 ppb	3 min	ICT-ESIPT Turn-on	10
BER9-HZ	2 steps	0.076 μM	20 min	ICT-ESIPT Turn-on	11
				ICT-ESIPT	

SPF-PA	＞5 steps	0.29 μM		Turn-on ICT-ESIPT Turn-on	
SP-CHO	3 steps	1.26 μM	10 min	ICT-ESIPT Turn-off	13
DMA	2 steps	11 nM	60 min	AIE Turn-on	14
CL-HZ	3 steps	9.3×10^{-10} M	60 min	ICT-ESIPT Turn-off	15

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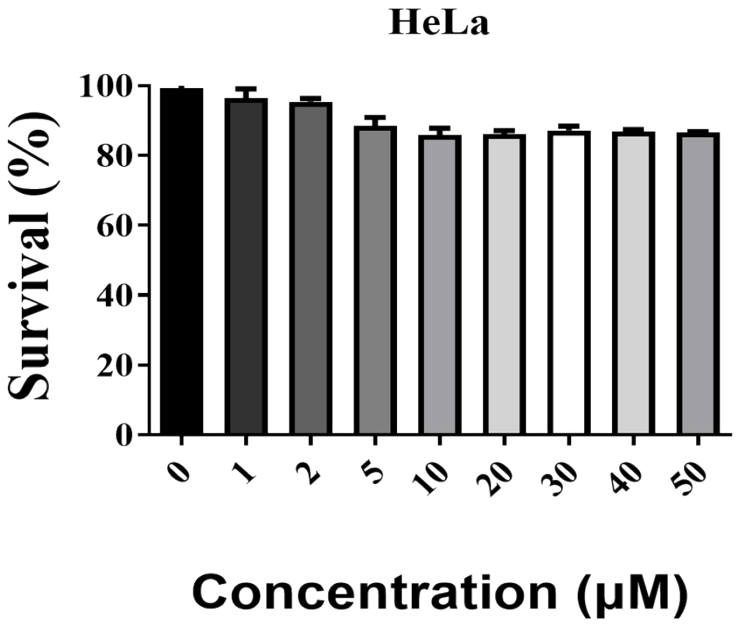

Fig. S5. At different concentrations of HFOAc (0 μM; 1 μM; 2 μM; 5 μM; 10 μM; 20 μM; 30 μM; 40 μM; 50 μM), the toxicity of HeLa cells.

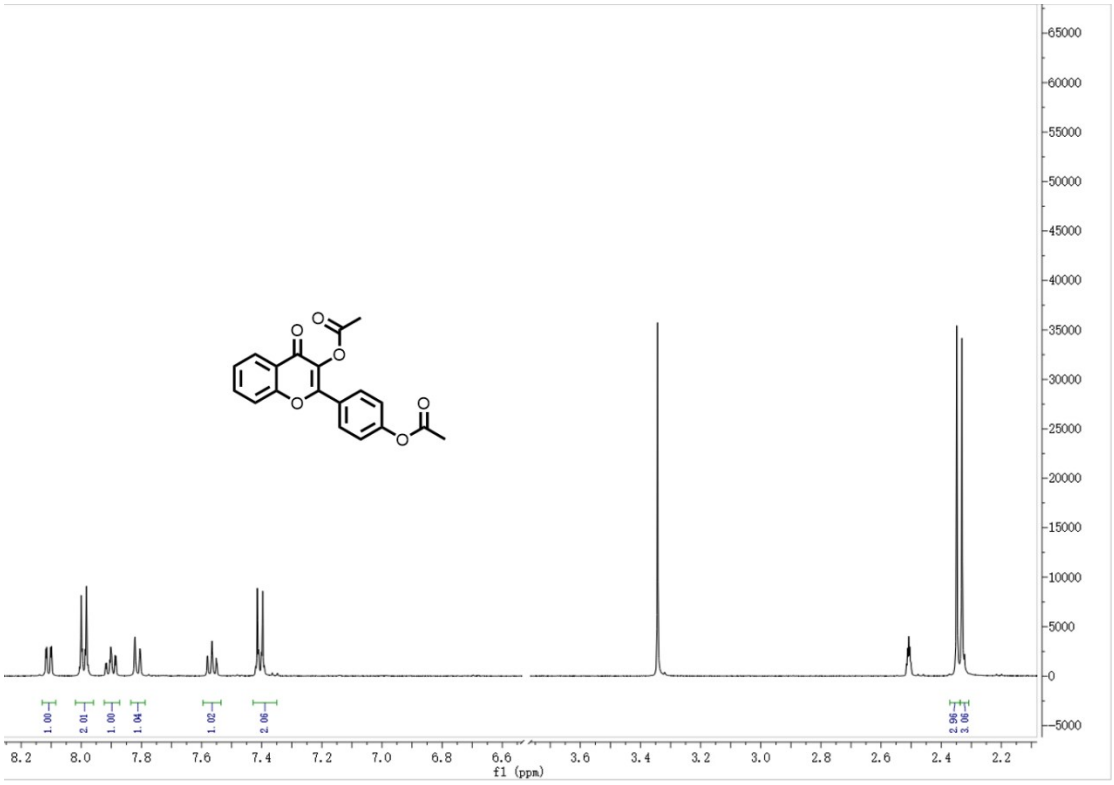

Fig. S6. ^{1}H NMR (DMSO-d_6) spectrum of probe HFOAc.

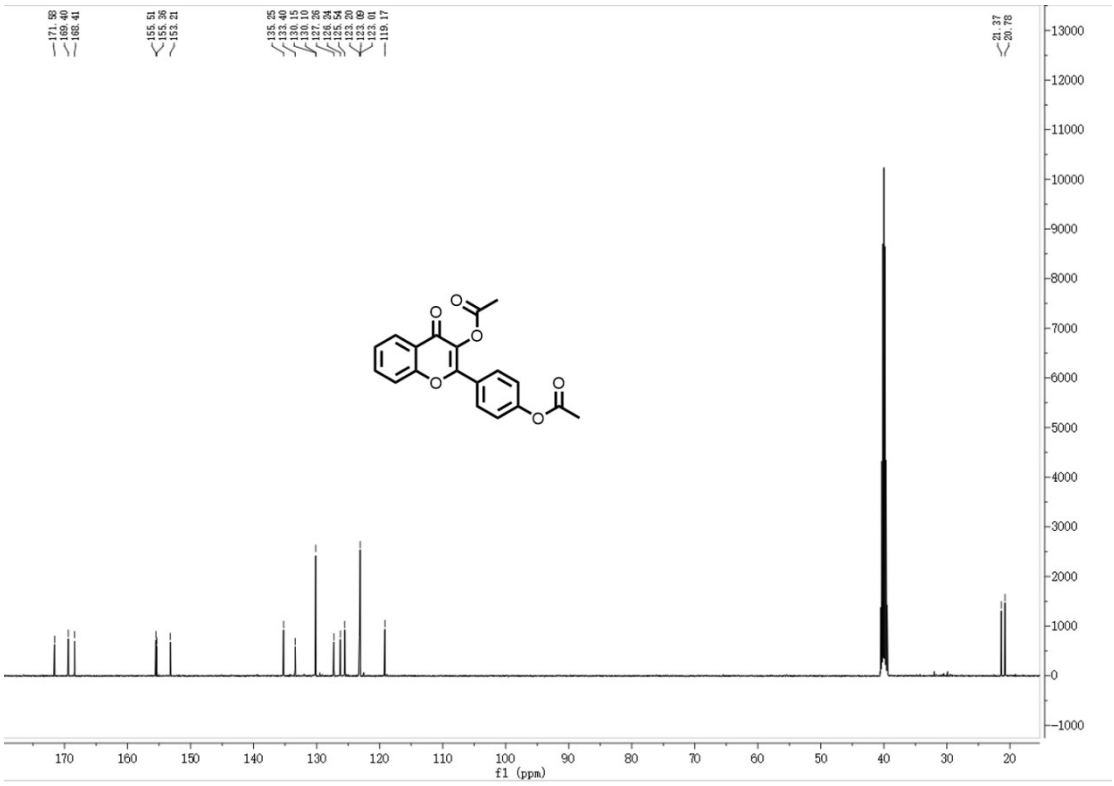

Fig. S7. ^{13}C NMR (DMSO-d_6) spectrum of probe HFOAc.

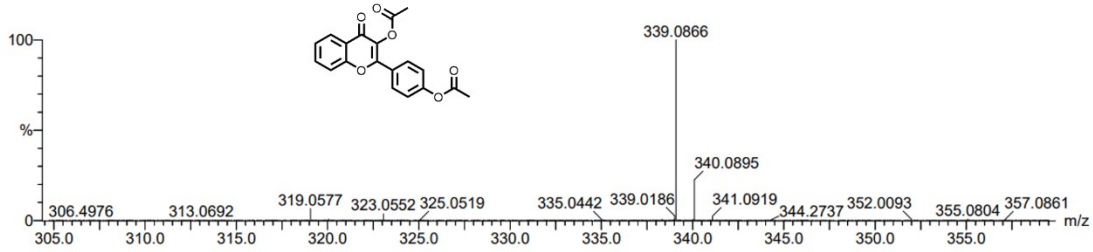

Fig. S8. HRMS (ESI) spectrum of probe HFOAc, $[M]^+$, 339.0866.

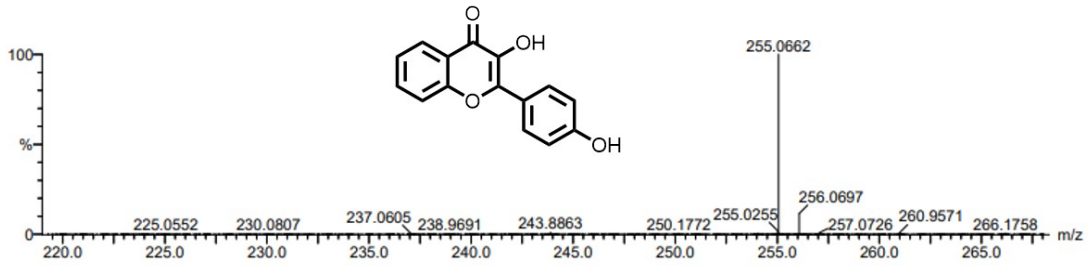

Fig. S9. HRMS (ESI) spectrum of the reaction products of probe HFOAc with N_2H_4, M^+, 255.0662.