

*Supporting Information for*

# Ethyl cyanoacetate based turn-on fluorescent probe for hydrazine and its bio-imaging and environmental applications

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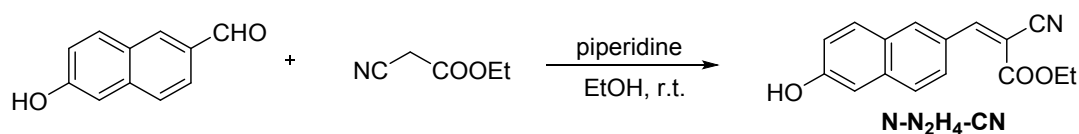
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## Materials and instruments

Unless otherwise noted, all reagents and materials were purchased from commercial company and used without further purification. Twice-distilled water was applied to all experiments. High-resolution electrospray (ESI-HRMS) mass spectra were examined from Bruker APEX IV-FTMS 7.0T mass spectrometer; NMR spectra were obtained from AVANCE III 400 MHz Digital NMR Spectrometer with TMS as an internal standard; Electronic absorption spectra were recorded on a LabTech UV Power spectrometer; Photoluminescent spectra were obtained with a HITACHI F4600 fluorescence spectrophotometer; The fluorescence images were collected with Nikon A1MP confocal microscopy with a CCD camera; The pH measurements were implemented on a Mettler-Toledo Delta 320 pH meter; analysis was exhibited on silica gel plates and column chromatography was carried out over silica gel (mesh 200-300). Both TLC and were purchased from the Qingdao Ocean Chemicals.

## Synthesis



A mixture of 6-hydroxy-2-naphthaldehyde (0.5 mmol, 86 mg, 1.0 equiv) and ethyl cyanoacetate (0.6 mmol, 67.8 mg, 1.2equiv) were dissolved in EtOH (3.0 mL) and piperidine (0.6mmol, 51.1mg, 1.2equiv) was added. After stirred at room temperature for 12 h, the reaction mixture was filtered by ether. The product was obtained to give the probe **N-N<sub>2</sub>H<sub>4</sub>** in the yield of 83%. <sup>1</sup>H NMR (400MHz, DMSO-*d*<sub>6</sub>) δ 10.47(s, 1H), 8.46(d, *J* = 15.2 Hz, 2H), 8.15(dd, *J*<sub>1</sub> = 1.6Hz,

$J_2 = 8.8\text{Hz}$ , 1H), 7.88(dd,  $J_1 = 10\text{ Hz}$ ,  $J_2 = 20$ , 2H), 7.21(s, 1H), 7.20(t,  $J = 10$ , 1H), 4.33(dd,  $J_1 = 7.2$ ,  $J_2 = 14.4$ , 2H), 1.32(t,  $J = 16$ , 3H).  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  163.3, 159.6, 156.1, 138.2, 136.3, 132.6, 128.1, 127.8, 126.8, 125.7, 121.0, 117.2, 110.2, 100.6, 63.14; HRMS (ESI)  $m/z$  calcd for  $\text{C}_{16}\text{H}_{13}\text{NO}_3$  (M+H) $^+$ : 268.0929; found 268.0973.

### **Preparation of Solutions of probe N-N<sub>2</sub>H<sub>4</sub> and Analytes**

Without other noted, all the tests were operated according to the following procedure. A stock solution (1.0 mM) of N-N<sub>2</sub>H<sub>4</sub> was prepared in DMSO. In a 10 mL tube the test solution of compounds N-N<sub>2</sub>H<sub>4</sub> was prepared by placing 0.09 mL of stock solution, 4.5 mL of DMSO, 4.5 mL of 0.1 M PBS buffer and an appropriate volume of N<sub>2</sub>H<sub>4</sub> sample solution. After adjusting the final volume to 10 mL with 0.1 M PBS buffer, standing at room temperature 3 min, 3 mL portion of it was transferred to a 1 cm quartz cell to measure absorbance or fluorescence. All fluorescence measurements were conducted at room temperature on a Hitachi F4600 Fluorescence Spectrophotometer. The slight pH variations of the solutions were achieved by adding the minimum volumes of NaOH (0.1 M) or HCl (0.2 M).

### **Vapor gas detection**

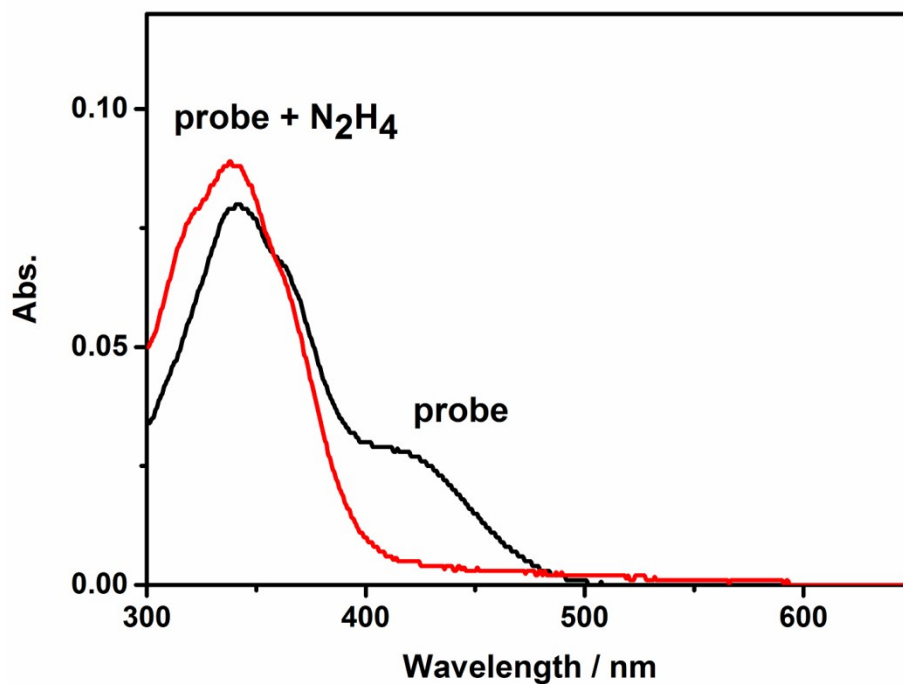
TLC plates were soaked in the solution of N-N<sub>2</sub>H<sub>4</sub> (0.1 mM, in DMSO). After dried, the N-N<sub>2</sub>H<sub>4</sub> probe-loaded TLC plates were placed to cover a flask containing different concentration of N<sub>2</sub>H<sub>4</sub> for 10 min at room temperature before observation.

### **Cytotoxicity assays**

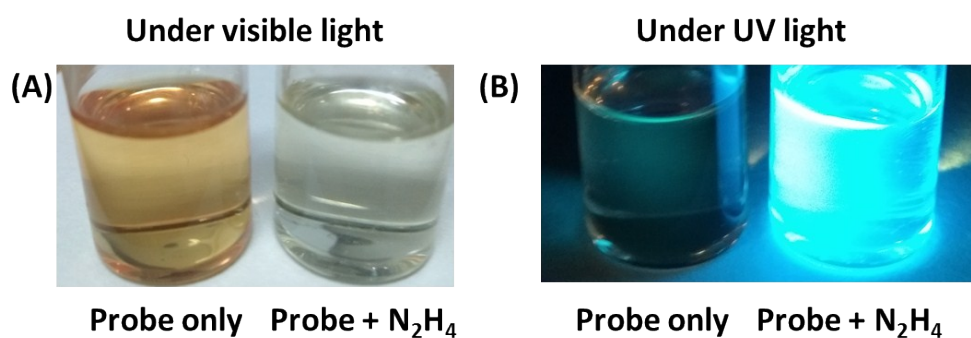
The living cells line were treated in DMEM (Dulbecco's Modified Eagle Medium) supplied with fetal bovine serum (10%, FBS), penicillin (100 U/mL) and streptomycin (100 µg/mL) under the atmosphere of CO<sub>2</sub> (5%) and air (95%) at 37 °C. The HeLa cells were then seeded into 96-well plates, and 0, 1, 5, 10, 20, 30 µM (final concentration) of the probe N-N<sub>2</sub>H<sub>4</sub> (99.9% DMEM and 0.1% DMSO) were added respectively. Subsequently, the cells were cultured at 37 °C in an atmosphere of CO<sub>2</sub> (5%) and air (95%) for 24 hours. Then the HeLa cells were washed with PBS buffer, and DMEM medium (100 µL) was added. Next, MTT (10 µL, 5 mg/mL) was injected to every well and incubated for 4 h. Violet formazan was treated with sodium dodecyl sulfate solution (100 µL) in the H<sub>2</sub>O-DMF mixture. Absorbance of the solution was measured at 570 nm by the way of a microplate reader. The cell viability was determined by assuming 100% cell viability for cells without N-N<sub>2</sub>H<sub>4</sub>.

### **Cell imaging**

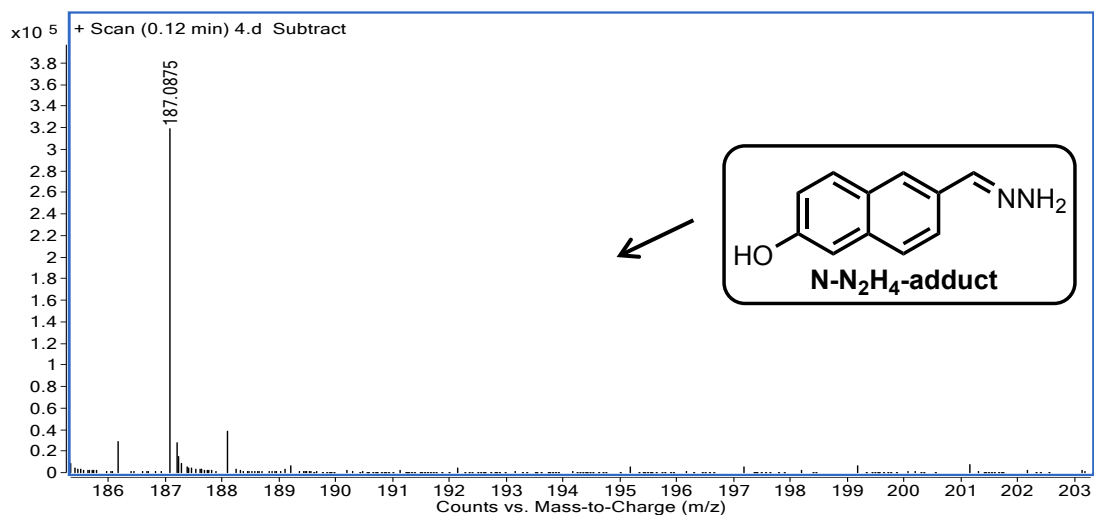
HeLa cells were grown in modified Eagle's medium (MEM) replenished with 10% FBS with the atmosphere of 5% CO<sub>2</sub> and 95% air at 37 °C for 24 h. The HeLa cells were washed with PBS when used. HeLa cells treated with N-N<sub>2</sub>H<sub>4</sub> (20.0 µM) for 30 min, then with N<sub>2</sub>H<sub>4</sub> (200.0 µM) for 30 min at 37 °C. The ideal fluorescence images were acquired with a Nikon A1MP confocal microscopy with the equipment of a CCD camera.



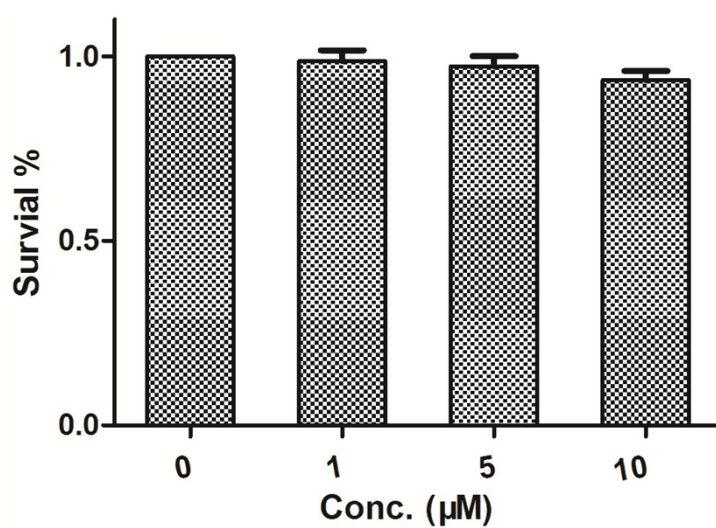
**Fig. S1.** The absorption spectra of  $N-N_2H_4$  ( $10 \mu M$ ) in pH 7.4 PBS/DMSO ( $v/v = 1/1$ ) in the absence or presence of  $N_2H_4$  (20 equiv).



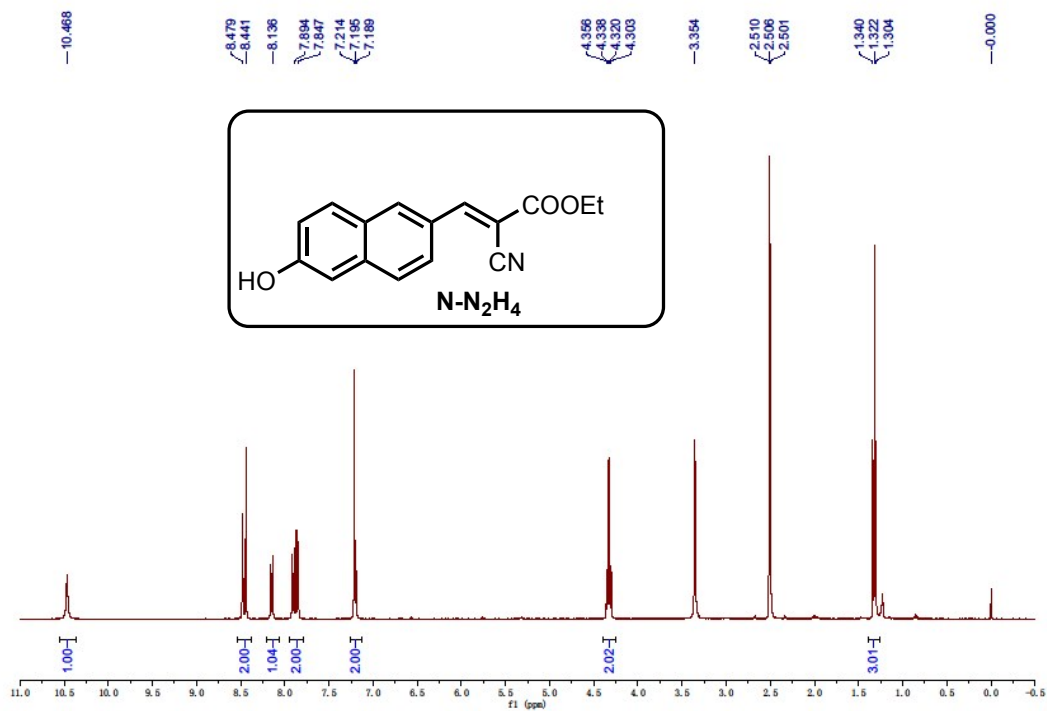
**Fig. S2** (A) The color changes of the probe  $N-N_2H_4$  ( $1 \text{ mM}$ ) in pH 7.4 PBS/DMSO ( $v/v = 1/1$ ) before and after added  $N_2H_4$  (200 equiv)) and (B) The fluorescence changes of the probe  $N-N_2H_4$  before and after added  $N_2H_4$  with 365 nm ultraviolet light.



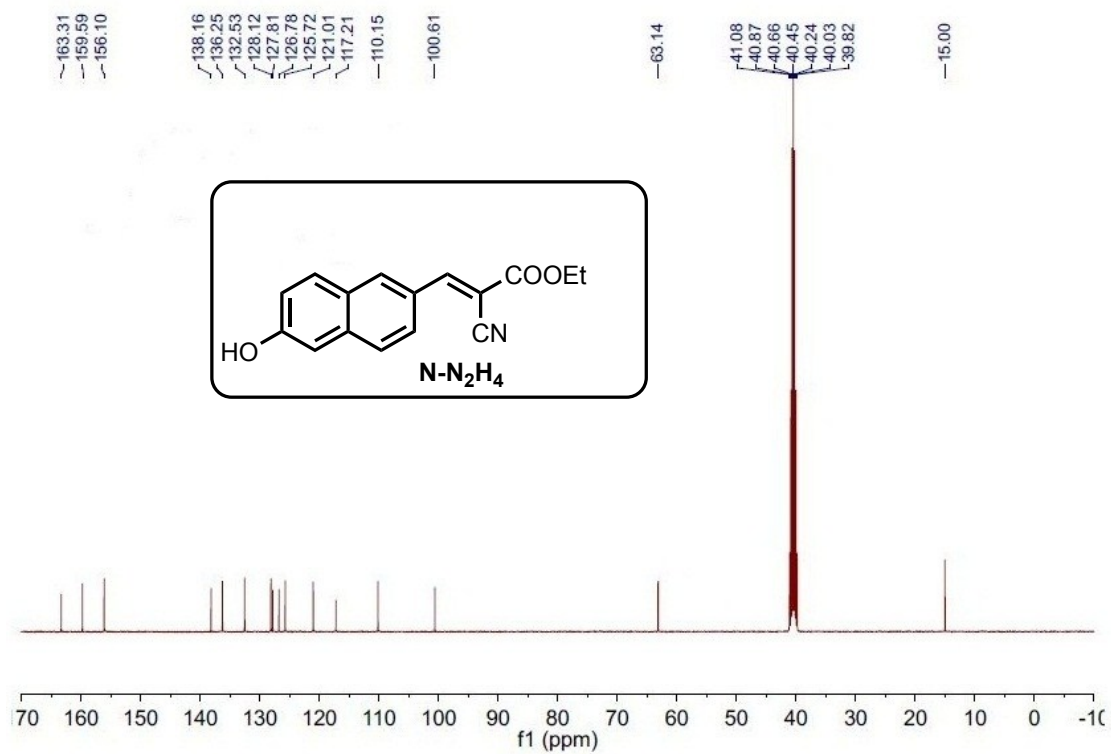
**Fig. S3.** HRMS (positive ion mode) spectrum of **N-N<sub>2</sub>H<sub>4</sub>** (20  $\mu$ M) after treatment with **N<sub>2</sub>H<sub>4</sub>** (400  $\mu$ M) in pH 7.4 PBS/DMSO (1: 1) for 60 min. The peak at m/z 187.0875 corresponds to **N-N<sub>2</sub>H<sub>4</sub>-adduct**.



**Fig. S4.** Cytotoxicity assays of **N-N<sub>2</sub>H<sub>4</sub>** at different concentrations (0  $\mu$ M; 1  $\mu$ M; 5  $\mu$ M; 10  $\mu$ M) for HeLa cells

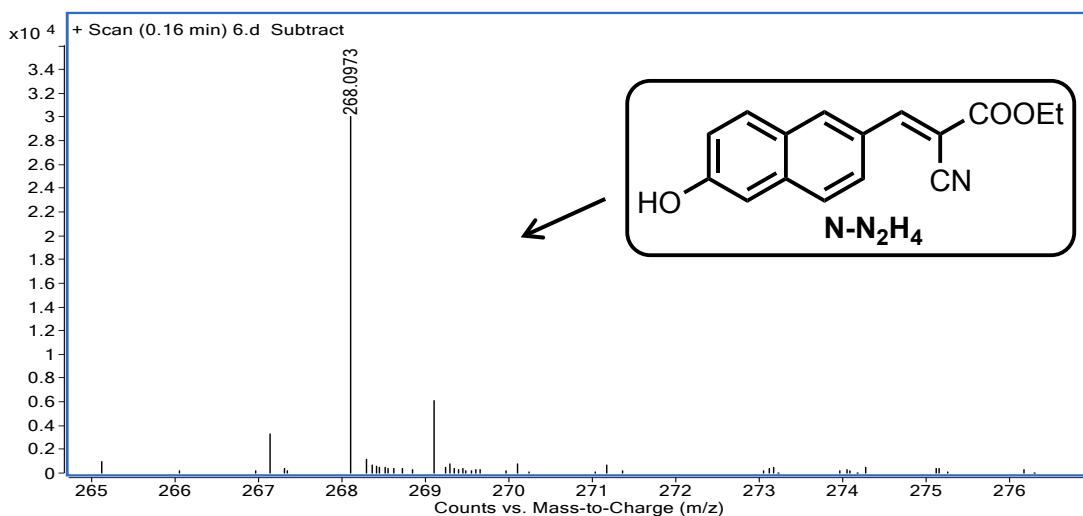


**Fig. S5.** <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) spectrum of **N-N<sub>2</sub>H<sub>4</sub>**.



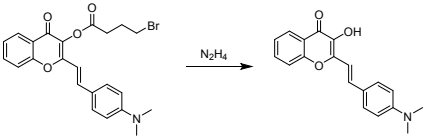
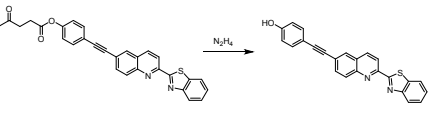
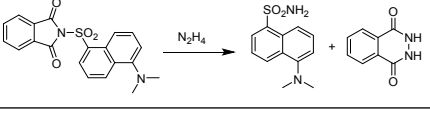
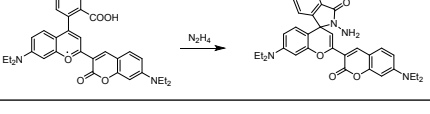
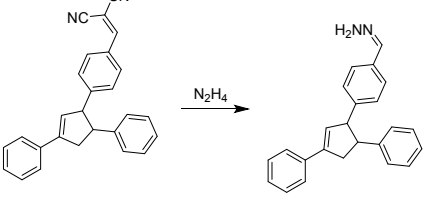
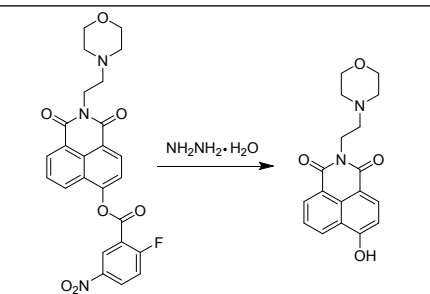
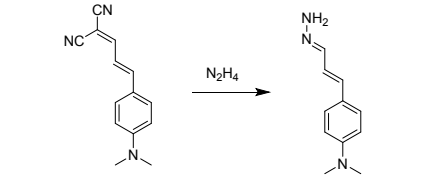
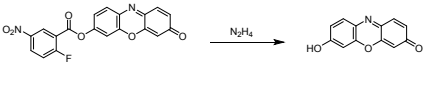
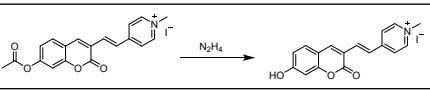
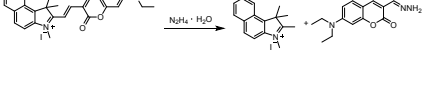
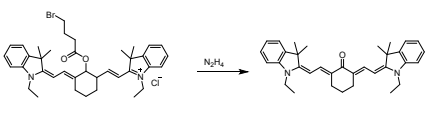
**Fig. S6.** <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) spectrum of **N-N<sub>2</sub>H<sub>4</sub>**.

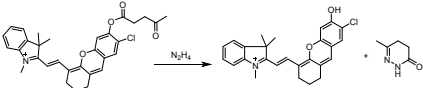
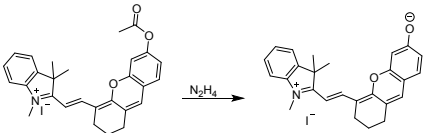




**Fig. S7.** HRMS spectrum of the probe **N-N<sub>2</sub>H<sub>4</sub>**.

Literature	Structure and sensing mechanism	Cell experiment	TLC plates
This Work		✓	✓
Xiaojun Peng et al, Org. Lett., Vol. 15, No. 15, 2013,4025		✓	×
Xuhong Qian et al, Org. Biomol. Chem., 2015, 13, 5344–5348		✓	×
B. Chen et al, Sensors and Actuators B 199 (2014) 93–100		×	×
X. Xia et al, Sensors and Actuators B 227 (2016) 411–418		✓	✓
D. Mandal et al, RSC Adv., 2015,1-10		✓	✓

Hongjun Zhu et al, Anal. Methods., 2016		×	×
S. Yu et al, Sensors and Actuators B 220 (2015) 1338–1345		✓	✓
Bao-Xiang Zhao, J. Mater. Chem. B, 2014, 2, 7344–7350		✓	×
X. Dai et al, Sensors and Actuators B 232 (2016) 369–374		✓	✓
X.-X. Zheng et al, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 138 (2015) 247–251		×	×
G. Feng et al, Anal. Methods, 2016		✓	✓
S.I. Reja et al, Sensors and Actuators B 222 (2016) 923–929		✓	✓
T. Tang et al, Chinese Chemical Letters 27 (2016) 540–544		✓	×
J. Yang et al, RSC Adv., 2016		✓	×
Zhonghai Ni et al, RSC Adv., 2017, 7, 25634–25639		×	×
Zhengliang.lu et al, Anal. Chem., 2017		✓	×

Weiyang Lin et al, Anal. Methods, 2013, 5, 3450–3453		✓	✗
Haixia Zhang et al, Anal. Chem., 2015		✓	✗

**Table S1.** Comparison of  $N-N_2H_4$  with other small molecular fluorescent hydrazine probes.