Supporting Information for

A Fluorescent Probe for Hydrazine and Its in Vivo Applications

Liangliang Xiao,a Jia Tu,a Shiguo Sun,a Zhichao Pei,a Yuxin Pei,a Yi Pangb and Yongqian Xu,*a

a College of Science, Northwest A&F University, Yangling, Shaanxi, P. R. China, 712100, xuyq@nwsuaf.edu.cn
b Department of Chemistry & Maurice Morton Institute of Polymer Science, The University of Akron, Akron, OH, 44325

Synthesis material and instruments

All chemicals and reagents were used directly as obtained commercially unless otherwise stated. All solvents were of reagent grade and water used was ultra filter deionized. Probe 4 was synthesized according to our previous work. S1 Absorption and emission spectra were collected by using a Shimadzu 1750 UV-visible spectrometer and a RF-5301 fluorescence spectrometer (Japan), respectively. NMR spectra were collected on a Bruker 500 avance III spectrometer. Chemical shifts (δ) were reported as ppm with TMS as the internal standard. Mass spectrometric (MS) data were obtained with HP1100LC/MSD MS and an LC/Q-TOF-MS instruments. The quantum yield of the sample was measured using quinine sulfate as the standard (Φ = 0.53, 0.1 M H2SO4). S2

Sample Preparation and Titration

Stock solutions of metal ions, anions, amino acids and amine complexes were prepared in deionized water. The concentration are fixed to 1.0×10^-2 M. Stock solution of fluorescent probes (5.0×10^-4 M) were prepared in CH3CN and then further diluted to 2.0×10^-5 M for titration experiments. Every time an appropriate volume of each analyte was added to the test solution. Excitation was provided at 390 nm.

Cell culture and fluorescence image

Hela cells were seeded on 35 mm glass-bottomed dishes (NEST) and incubated in RPMI-1640 in an incubator (37 °C, 5% CO2 and 20% O2) for 24 h. The cells were rinsed slightly 3 times with fresh RPMI-1640 and incubated in RPMI-1640 medium spiked with or without sensor (5 μM) for 30 min, respectively. After washing with fresh RPMI-1640, the cells treated with sensor were further incubated in fresh RPMI-
1640 containing of 50 μM hydrazine for 0.5 h. Cells were then analyzed by Laser Scanning Confocal Microscope (A1R).

**Synthesis routine**

![Chemical synthesis diagram]

1,4-Bis(ethyoxyl)benzene (5)

A mixture of 1,4-hydroquinone (4.40 g, 40 mmol), iodoethane (10.00 mL, 120 mmol), and K$_2$CO$_3$ (22.00 g, 160 mmol) in acetonitrile (100 mL) was heated to reflux for two days. After cooling to room temperature, the precipitates were filtered, then evaporation of the solvent under reduced pressure. The solid residues were collected and purified on a silica gel column by using an eluent (petroleum ether), the product 5 was obtained as a white solid (4.90 g, 75.00% yield).

2,5-Bis(bromomethyl)-1,4-bis(ethyoxyl)benzene (6)

HBr (10 mL, 30 wt % in acetic acid) was added to a suspension of 5 (4.00 g, 23 mmol) and paraformaldehyde (1.40 g, 46 mmol) in acetic acid (80 mL). The mixture
was heated to 60 °C, and then stirred for 3 h. As the reaction proceeded, the suspension changed to clear solution first and then became a thick suspension again. After cooling to room temperature, the suspension was poured into water (400 mL). The precipitates were filtered and washed with water (3×10 mL). Then dried under vacuum and the crude product 6 was obtained as an off-white solid (6.70 g, 78.0% yield).

2,5-Bis(ethyoxyl)benzene-1,4-dialdehyde (7)

A solution of 2 (2.50 g, 7.70 mmol), potassium acetate (2.50 g, 23.10 mmol), and tetra(n-butyl)ammonium bromide (0.30 g) in a mixture of acetonitrile (50 mL) and chloroform (25 mL) was heated to reflux for 8 h. The mixture was washed with 100 mL water, and organic solution was removed on a rotary evaporator. The solid residues were dissolved in a 1:1 mixture of THF and MeOH, then a solution of NaOH (1.00 g, 25 mmol) and H₂O (2 mL) was added dropwise to this solution. This mixture was heated to reflux for 8 h. After cooling to room temperature, the solvent was removed, the solid residues were washed with water to remove the salt, then dried under vacuum. The dried crude product was dissolved in 20 mL CH₂Cl₂. The solution was added dropwise to a stirred solution of freshly prepared pyridium chlorochromate (6.30 g, 29.30 mmol) in CH₂Cl₂ (200 mL) at room temperature. After addition was completed, stirring was continued for an additional 3 h. The reaction mixture was then directly transferred onto the top of a short silica gel column. The yellow and highly fluorescent compound was then washed off the column with CH₂Cl₂/petroleum ether (1:2), affording product 7 (1.10 g, 61.50% yield). ¹H NMR (500 MHz, CDCl₃) δ = 10.56 (d, J = 1.2 Hz, 2H), 7.46 (s, 2H), 4.21 (q, J = 7.0 Hz, 4H), 1.51 (t, J = 7.0 Hz, 6H). ¹³C NMR (125 MHz, CDCl₃) δ = 189.49, 155.08, 129.27, 111.69, 64.85, 14.65.

2,5-bis(ethyoxyl)-4-(benzoxazolyl) benzaldehyde (3)

A solution of o-aminophenol (0.24 g, 2.25 mmol) in methyl alcohol (20 mL) was added dropwise to a stirred solution of compound 7 in methyl alcohol (10 mL) at 70 °C. After addition was completed, stirring was continued for an additional 5 h. Then the solvent was removed using rotary evaporator. After that the solution of DDQ (0.50 g, 2.25 mmol) in 150 mL CH₂Cl₂ was added dropwise to the above residue. The reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was treated with 100 mL saturated Na₂CO₃, then washed with water, and dried over
Na₂SO₄. After evaporation of the solvent, the solid residues were collected and purified on a silica gel column by using an eluant (petroleum ether: CH₂Cl₂=1:1), the product 3 was obtained as a yellow solid (0.15 g, 21.42% yield). ¹H NMR (500 MHz, CDCl₃) δ = 10.56 (d, J=1.2 Hz, 1H), 7.93-7.79 (m, 2H), 7.73-7.60 (m, 1H), 7.55 (s, 1H), 7.49-7.38 (m, 2H), 4.35-4.19 (m, 4H), 1.55 (t, J=7.0 Hz, 6H). ¹³C NMR (125 MHz, CDCl₃) δ = 189.28, 161.03, 155.16, 151.79, 150.86, 141.67, 127.08, 125.62, 124.70, 122.97, 120.37, 115.65, 112.54, 110.78, 65.69, 65.07, 14.77, 14.75.

Synthesis of probe 1

A solution of compound 3 (0.20 g, 1.60 mmol) in 10 mL of dry CH₂Cl₂ was cooled to -30°C with ice/ethanol alcohol. BBr₃ (0.30 mL) in 10 mL CH₂Cl₂ was added dropwise under a nitrogen atmosphere. The reaction mixture was stirred at -30°C for 1 h and then at room temperature for 24 h. Following the addition of 4.00 mL distilled H₂O, the reaction mixture was stirred for additional 2 h and poured into a mixture of water (20 mL) and CH₂Cl₂ (20 mL). The organic layer was separated, and the aqueous layer was extracted twice with CH₂Cl₂ (30 ml). The combined organic layer was washed with brine and dried over anhydrous MgSO₄. After removal of the solvent on a rotary evaporator, the solid residues were collected and purified on a silica gel column by using an eluent (petroleum ether/CH₂Cl₂, 1:1), the product probe 1 was obtained as a yellow solid (0.10 g, 62.5% yield). ¹H NMR (500 MHz, DMSO-d₆) δ = 10.59 (s, 1H), 10.47 (s, 1H), 10.31 (s, 1H), 7.87 (d, J = 7.7 Hz, 2H), 7.63 (s, 1H), 7.46-7.53 (m, 2H), 7.26 (s, 1H). ¹³C NMR (125 MHz, DMSO-d₆) δ = 190.41, 161.46, 153.35, 150.27, 149.61, 140.10, 126.91, 126.35, 125.94, 120.14, 117.42, 115.95, 115.31, 111.70. m/z (TOF-LD): Calcd. [M+H]⁺ For C₁₄H₉NO₄: 256.0610, found: 256.0600.

Synthesis of probe 2

A solution of probe 1 (0.20 g) in anhydrous DMF (5 mL) was treated with K₂CO₃ (0.12 g) at 25 °C, and the mixture was stirred for 30 min. Ethyl iodide (0.10 g) was added, and the reaction mixture was further stirred for 20 h before being quenched by the addition of H₂O (100 mL). The aqueous layer was extracted with CH₂Cl₂ (2×100 mL). The combined organic layer was dried over Na₂SO₄ and concentrated under vacuum. The product was isolated by flash chromatography on silica gel by using an eluent (petroleum ether/CH₂Cl₂, 1:1), the product probe 2 was obtained as a yellow solid (0.15 g, 65.0% yields). ¹H NMR (500 MHz, CDCl₃) δ ppm 10.48 (s, 1H), 7.71
$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ ppm 189.11, 161.58, 153.79, 152.29, 149.23, 139.90, 128.56, 126.25, 125.43, 119.73, 116.30, 115.35, 110.79, 110.35, 65.00, 14.70. m/z (TOF-LD): Calcd. [M+H]$^+$ For C$_{16}$H$_{13}$NO$_4$: 284.0923, found: 284.0919.

**Fig. S1** The fluorescence spectra of probe 2 (20 $\mu$M) in CH$_3$OH and THF, $\lambda_{ex}$=390 nm. The fluorescent bands at 470 and 580 nm are attributed to *enol* and *keto* emission, which is consistent with ESIPT characteristic.

**Fig. S2** UV-Vis absorption (a) and fluorescence spectra (b) of probe 1 (20 $\mu$M) upon addition of N$_2$H$_4$ in CH$_3$CN ($\lambda_{ex}$=390 nm). Inset: The fluorescence color change before and after addition of hydrazine excited by hand-held UV lamp. Each spectrum was recorded after 50 min of reaction.
**Fig. S3** Time-dependent fluorescence intensity of probe 1 (20.0 μM) upon addition of NH$_2$NH$_2$ (0.2 mM) and NH$_2$OH (0.2 mM) in CH$_3$CN/HEPES Buffer (1:2, v/v, pH 7.4), $\lambda_{ex}=390$ nm.

**Fig. S4** The fluorescence intensity change of probe 1 (20 μM) at 560 nm response to the different value of pH.
**Fig. S5** The $^1$H NMR spectra titration of probe 1 in DMSO-$d_6$/D$_2$O (10:1) upon addition of N$_2$H$_4$. Inset: the enlarged spectra in the region of 11-10.

**Fig. S6** HRMS spectrum of the reaction product of probe 1 with hydrazine.
**Fig. S7** HOMO–LUMO energy levels and interfacial plots of the orbitals for probe 1 and the corresponding product.

![HOMO–LUMO plots](image)

**Fig. S8** The optimizational molecular structures for probe 1 (a) and the corresponding product (b).

![Molecular structures](image)
Table S1 The calculated data for probe 1

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Table S2 The calculated data for the corresponding product

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Fig. S9 Time-dependent fluorescence intensity of probe (20.0 μM) was treated with hydrazine (0.2 mM) (a) in CH$_3$CN/HEPES Buffer (1:2, v/v, pH 7.4) and (b) in SDS solution (10 mM), $\lambda_{ex}$=390 nm. c) Schematic diagram of micelle-induced enrichment of hydrazine on micelle surface, conductive to reaction with probe.
Fig. S10 Fluorescence emission spectra of (a) probe 2 (20 μM), (b) probe 3 (20 μM) and (c) probe 4 (20 μM) upon addition of N₂H₄ in CH₃CN/HEPES Buffer (1:2, v/v, pH 7.4), λₑₓ=390 nm. Each spectrum was recorded after 15 min of reaction.

Fig. S11 The fluorescence spectra response of probe 1 (20μM) (a) and probe 2 (20μM) (b) with time duration in the presence of 100 μM hydrazine in cationic CTAB (5 mM) micelles solution, λₑₓ=390 nm.

Probe 1 and 2 showed no obvious fluorescence spectra change with increasing time upon to 10 equivalents of hydrazine in cationic cetyltrimethyl ammonium bromide (CTAB) micelles (5 mM) solution. These phenomena are unlike that in anionic SDS micelles solution (Fig. S8b). It is presumed that cationic CTAB micelles-encapsulated probe cannot access to positively charged hydrazine (pKₐ=14.9) because of electrostatic repulsion.
Fig. S12 Fluorescent color changes of probe 1 (20 μM)-coated filter paper after exposure to an excess quantity of various vapors, including hydrazine, ammonia, methylamine, n-butylamine, formaldehyde, dimethylamine, H₂O₂, HCl, and CO₂ for 10 min, respectively. The fluorescent color changes were observed using a hand-held UV lamp with an excitation at 365 nm.
The $^1$H NMR spectrum of compound 7

PROTON CDC13 \( \text{(D:\2014-1) ZHL 18} \)

The $^{13}$C NMR spectrum of compound 7

C13CPD CDC13 \( \text{(D:\2014-1) ZHL 18} \)
The $^1$H NMR spectrum of probe 3

The $^{13}$C NMR spectrum of probe 3
The $^1$H NMR spectrum of probe 1

PROTON DMSO (D:\2014-1) ZHL 19

The $^{13}$C NMR spectrum of probe 1

C13CPD DMSO (D:\2014-1) ZHL 19
The HRMS spectrum of probe 1

The $^1$H NMR spectrum of probe 2

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The $^{13}$C NMR spectrum of probe 2

The HRMS spectrum of probe 2