Supporting Information

Hydrazine Detection in Gas–state and Aqueous Solution Based on Gabriel Mechanism and Its Imaging in Living Cells

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1. Materials and General Methods.
   Silica gel P60 (Qingdao) was used for column chromatography. All chemicals were purchased from Sigma-Aldrich or Aldrich and were used as received. 1H-NMR spectra
were collected in CDCl$_3$ and DMSO (Cambridge Isotope Laboratories, Cambridge, MA) at 25 ºC on a Bruker AV-400 spectrometer at NMR Facility of East China University of Science and Technology (ECUST). All chemical shifts are reported in the standard δ notation of parts per million using the peak of residual proton signals of CDCl$_3$ or DMSO as an internal reference. Electrospray ionization (ESI) analyses were performed in the Mass Instrumentation Facility of Analysis & Research Center of ECUST by using a Voyager RP MALDI-TOF spectrometer (Micromass LCTTM, Mass Spectrometry Instruments Ltd.).

2. Synthesis

4-Amino-N-(n-butyl)naphthalimide (2). A mixture of 3 (400 mg, 1.88 mmol) and 1-Butanamine (411.7 mg, 3.01 mmol) in ethanol (25 mL) in a single necked flask and was heated to reflux for 4 hours. After cooling to room temperature, The solvent was removed under reduced pressure and purification by flash column chromatography as a yellow solid (460 mg, 91.45 % yield). $^1$H-NMR (DMSO-$d_6$, 400 MHz): δ 8.57 (1H, d, J = 8.32 Hz), 8.39 (1H, d, J = 7.2 Hz), 8.15 (1H, d, J = 8.32 Hz), 7.62 (1H, t, J = 7.52 Hz), 7.41 (2H, s), 6.81 (1H, d, J = 8.36 Hz), 3.98 (2H, t, J = 7.12 Hz), 1.55 (2H, m, J = 7.36 Hz), 1.30 (2H, m, J = 7.36 Hz) and 0.89 (3H, t, J = 7.32 Hz). $^{13}$C-NMR (DMSO-$d_6$, 100 MHz): δ 164.2, 163.4, 153.1, 134.4, 131.4, 130.1, 129.7, 124.4, 122.3, 119.8, 108.6, 108.0, 30.3, 20.3, 14.2.

4-phtalamide-N-(n-butyl)naphthalimide (1). A mixture of 2 (500 mg, 1.86 mmol) and phthalic anhydride (331 mg, 2.24 mmol) in acetic acid (15 mL) then heated to reflex and stirred for 4 hours. The solvent was removed under reduced pressure. Purification by flash column chromatography (silica gel, 1:4 hexane/EtOAc) gave 1 as an white solid (620 mg, 83.5 % yield). $^1$H NMR (CDCl$_3$, 400 MHz) δ 8.72 (d, J = 7.74 Hz, 1H), 8.66 (dd, J = 7.24, 0.85 Hz, 1H), 8.07-8.02 (m, 2H), 8.00 (dd, J = 8.48, 0.84 Hz, 1H), 7.92-7.87 (m, 2H), 7.81-7.70 (m, 2H), 4.25-4.18 (m, 2H), 1.74 (td, J = 15.23, 7.59 Hz, 2H), 1.46 (t, J = 14.71, 7.35 Hz, 2H), 0.99 (t, J = 7.35 Hz, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz) δ: 167.1, 163.9, 163.5, 135.0, 134.0, 131.7, 130.9, 129.1, 128.8, 127.8, 127.7, 124.3, 123.3, 40.4, 30.2, 20.4, 13.9. HREI-MS: calcd for C$_{26}$H$_{23}$N$_3$O$_9$ [M]+ 398.1268, found 398.1271.
3. Spectroscopic Materials and Methods & Determination of quantum yield

Double distilled water was used to prepare all aqueous solutions. All spectroscopic measurements were performed in water and DMSO (V/V = 6:4) at r.t. All pH measurements were made with a Sartorius basic pH-Meter PB-10. Absorption spectra were recorded using a Varian Cary100 Bio UV-Visible spectrophotometer. Fluorescence spectra were recorded using a Varian Cary Eclipse scanning spectrofluorometer equipped with a Xenon flash lamp. Samples for absorption and fluorescence measurements were contained in 1 cm×1 cm quartz cuvettes (3.5 mL volume). All cell images were taken Olympus IX51 with Xenon lamp and Olympus digital camera.

Determination of quantum yield

The quantum yield of sensor RHP and RHF were determined according to the literature.

\[
\Phi = \frac{\Phi _B I_B A_B \lambda _{exB} \eta _B}{I_A A_1 \lambda _{exA} \eta _A}
\]

Where \( \Phi \) is quantum yield; \( I \) is integrated area under the corrected emission spectra; \( A \) is absorbance at the excitation wavelength; \( \lambda _{ex} \) is the excitation wavelength; \( \eta \) is the refractive index of the solution; the subscripts 1 and B refer to the unknown and the standard, respectively. We chose Fluorescein with 0.1 M NaOH solution as standard, which has the quantum yield of 0.95.\(^1\) The quantum yields of RHP and RHF were calculated as 0.112 and 0.364, respectively.

<table>
<thead>
<tr>
<th>Compds</th>
<th>( \lambda _{abs} ) (nm)</th>
<th>( \varepsilon ) [M(^{-1}) cm(^{-1})]</th>
<th>( \lambda _{em} ) (nm)</th>
<th>( \Phi _f )( ^{[a]} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>340</td>
<td>6600</td>
<td>540</td>
<td>0.36</td>
</tr>
<tr>
<td>2</td>
<td>435</td>
<td>8800</td>
<td>540</td>
<td>0.11</td>
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\( ^{[a]} \) Ref.1

**Fig. S1.** Absorption (left) and fluorescent emission (right) spectra of 1 and 2 (Both concentration of 1 and 2 are 100 μM) water and DMSO (v/v = 4:6) at r.t.

![Absorption and fluorescent emission spectra](image)

**Fig. S2.** The pH titration of compounds probe 1 in water (contains 60 % DMSO)

![pH titration of compounds probe 1](image)

### 4. Hydrazinolysis sensitivity assay.

**Fig. S3.** The fluorescence intensity collected at 540 nm for probe 1 (50.0 μM in H₂O/DMSO = v / v, 4: 6) to different hydrazine concentration. All spectra were acquired 15 min after hydrazine addition and obtained with excitation at 380 nm.

![Fluorescence intensity vs. hydrazine concentration](image)
5. Kinetic analysis of probe 1 to hydrazine

**Fig. S4.** Time-dependent fluorescence intensity (50.0 μM) was treated with hydrazine (0.5 mM) in H₂O/DMSO = v / v, 4: 6) with λ_ex = 380 nm

6. Selectivity of probe 1 to hydrazine and other species

**Fig. S5.** Fluorescence responses of probe 1 (50 μM) to hydrazine and other species. Each spectrum was recorded after 2 min of reaction in a mixture of water and DMSO (4 : 6, v/v) at r.t. to various representative species and primary amines (500 μM)
Fig. S6. Fluorescence responses of probe 1 (50 μM) to hyarazine and other species. Each spectrum was recorded after 2 min of reaction in a mixture of water and DMSO (4 : 6, v/v) at r.t. to various representative species and primary amines (5 mM).

Fig. S7. Fluorescence responses of probe 1 (50 μM) to hyarazine and other species. Each spectrum was recorded after 2 min of reaction in a mixture of water and DMSO (4 : 6, v/v) at r.t. to various representative species and primary amines (0.5 mM): 1. blank; 2. Hg²⁺; 3. Co³⁺; 4. Cr³⁺; 5. Cd²⁺; 6. Fe³⁺; 7. Ni²⁺; 8. Zn²⁺; 9. Cu²⁺; 10. Al³⁺; 11. Mg²⁺; 12. Ca²⁺; 13. Ag⁺; 14. hydrazine.
Fig. S8. Fluorescence responses of probe 1 (50 μM) to hydrazine and other species. Each spectrum was recorded after 2 min of reaction in a mixture of water and DMSO (4:6, v/v) at r.t. to various representative species and primary amines (0.5 mM): 1. blank; 2. Cl−; 3. B’r; 4. I−; 5. SO4^{2−}; 6. SO3^{2−}; 7. ClO4^{−}; 8. HCO3^{−}; 9. SCN^{−}; 10. HPO4^{2−}; 11. Hydrazine.

7. Preparation of Cell Culture

Hela cells were seeded in a 6-well tissue culture dish at the Cell Culture Facility of Shanghai Normal University (SHNU) and differentiated for 36 h in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS).

8. Hydrazine imaging in live-cell assay

Hela cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C and under 5% CO2 in a CO2 incubator. Cells were typically passaged with sub-cultivation ratio of 1:4 every two days. After incubation with the 1, the media was removed and the cells were washed twice with phosphate buffered saline (PBS, pH 7.0 at 37°C containing 1% DMSO) to remove excess extracellular dye. Fluorescence imaging was performed with an Olympus IX51 with Xenon lamp and Olympus digital camera. Green emission was collected with a 540-580 nm window. In each experiment we set the same exposure time (1/5 s). (1)
9. NMR spectra

$^1$H - Compound RHF:

$^{13}$C - Compound RHF:
HR-EI(+)