Supporting Information

Molecular Design and Synthesis of a pH Independent and Cell Permeant Fluorescent Dye and its Applications

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Table S1 Absorbance and emission properties of the dye 1 in different solvents.

<table>
<thead>
<tr>
<th>solvents</th>
<th>λ&lt;sub&gt;abs(ε)&lt;/sub&gt;</th>
<th>λ&lt;sub&gt;em&lt;/sub&gt;</th>
<th>solvents</th>
<th>λ&lt;sub&gt;abs(ε)&lt;/sub&gt;</th>
<th>λ&lt;sub&gt;em&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-TFA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+TFA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-TFA</td>
<td>+TFA</td>
<td>λ&lt;sub&gt;em&lt;/sub&gt;</td>
</tr>
<tr>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;8&lt;/sub&gt;</td>
<td>582(200)</td>
<td>582(45800)</td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;CN</td>
<td>576(400)</td>
<td>576(52400)</td>
</tr>
<tr>
<td>CH&lt;sub&gt;2&lt;/sub&gt;Cl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>581(2400)</td>
<td></td>
<td>CH&lt;sub&gt;3&lt;/sub&gt;OH</td>
<td>568(12000)</td>
<td>579(63000)</td>
</tr>
<tr>
<td>THF</td>
<td>--&lt;sup&gt;c&lt;/sup&gt;</td>
<td>582(29700)</td>
<td>EtOH</td>
<td>575(1100)</td>
<td>581(68500)</td>
</tr>
<tr>
<td>AcOEt</td>
<td>579(100)</td>
<td>579(42800)</td>
<td>DMSO</td>
<td>592(1500)</td>
<td>587(39700)</td>
</tr>
<tr>
<td>CHCl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>--</td>
<td>581(64000)</td>
<td>DMF</td>
<td>--</td>
<td>584(46300)</td>
</tr>
<tr>
<td>C&lt;sub&gt;3&lt;/sub&gt;H&lt;sub&gt;6&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>568(1000)</td>
<td>578(7600)</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>572(38100)</td>
<td>576(38400)</td>
</tr>
<tr>
<td>C&lt;sub&gt;6&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;O</td>
<td>578(300)</td>
<td>578(47800)</td>
<td></td>
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</tbody>
</table>

Notes: a: “-TFA”without TFA; b: “+TFA”with 1% TFA; c: “--” means the absorbance value is negative.
**Fig. S1** Absorption spectra of 1 (10 µM) in different pH solution. a) Absorption spectra of 1 under different pH conditions in water. b) Absorbances of 1 at 578 nm under different pH conditions. c) The pKa (3.01) of the interconversions between cationic and neutral forms derived from the absorbance at 578 nm. d) The pKa (10.0) of the interconversions between neutral and anionic forms derived from the absorbance at 578 nm.

**Fig. S2** Emission spectra of the dye 1 (5 µM) in different pH solution. a) Emission spectra of 1 under different pH conditions in water. b) Fluorescence intensities of 1 at 617 nm under different pH conditions. c) The pKa (3.4) of the interconversions between cationic and neutral forms derived from the emission intensities at 617 nm. d) The pKa (10.1) of the interconversions between neutral and anionic forms derived from the emission intensities at 617 nm (λ_ex = 540 nm, slit = 5 nm, 10 nm).
**Fig. S3** Images of L929 cells incubated with the dye 1 (15 µM). a) and b) fluorescence and brightfield image after staining for 45 min. c) and d) fluorescence and brightfield image after staining for 24 h.

![Fig. S3 Images of L929 cells incubated with the dye 1 (15 µM). a) and b) fluorescence and brightfield image after staining for 45 min. c) and d) fluorescence and brightfield image after staining for 24 h.](image)

**Fig. S4** Absorbance of the hydrazide 5 (5 µM) at varied concentrations of Hg^{2+} in EtOH/H_{2}O (1:3, v/v).

![Fig. S4 Absorbance of the hydrazide 5 (5 µM) at varied concentrations of Hg^{2+} in EtOH/H_{2}O (1:3, v/v).](image)

**Fig. S5** Color changes of 5 (1 × 10^{-5} M) in the presence of 5.0 equiv of various ions in ethanol / water (v / v; 1:3).

![Fig. S5 Color changes of 5 (1 × 10^{-5} M) in the presence of 5.0 equiv of various ions in ethanol / water (v / v; 1:3).](image)
Figure S6. HRMS spectra of the reaction mixture of 5 with Hg$^{2+}$. The peak (m/z) at 452.1981 corresponds to the chemosensor 5 \([5 + H]^+\) ion (Calcd: 452.1969). The peak (m/z) at 438.1716 corresponds to \([1 + H]^+\) ion (Calcd: 438.1705).

Thus, we suggested that the selective optical responsive mechanism was the Hg$^{2+}$-promoted hydrolysis as shown in the following scheme.

Fig. S7 Emission (at 621 nm) of the hydrazide 5 at different concentrations of Hg$^{2+}$ (0, 0.004, 0.008, 0.012, 0.016, 0.02, 0.024 μM) added, normalized between the minimum emission (0.0 μM Hg$^{2+}$) and the emission at 0.023 μM Hg$^{2+}$. The fluorescence intensities were linearly proportional to the amount of Hg$^{2+}$ in this range, and this demonstrated the detection limit is as sensitive as $2.89 \times 10^{-9}$ M.
**Fig. S8** Job’s plot of the interactions between 5 and Hg$^{2+}$ in EtOH/water (1:3, v/v) showing the 1:1 stoichiometry. Total concentration of 5 and Hg$^{2+}$ was kept constant at 10.0 μM. Emission intensity is recorded at 621 nm.

**Fig. S9** UV-vis spectra of the hydrazide 5 (10 μM) in the presence of nitrate salts (5.0 equiv) of Na$^+$, K$^+$, NH$_4^+$, Ag$^+$, Mg$^{2+}$, Ca$^{2+}$, Pb$^{2+}$, Hg$^{2+}$, Co$^{2+}$, Cd$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Cu$^{2+}$, Cr$^{3+}$, Fe$^{2+}$, Fe$^{3+}$, and Al$^{3+}$ in ethanol/H$_2$O (v/v, 1:3).
Fig. S10. $^1$H NMR of 1 (400 MHz, DMSO-d$_6$).

Fig. S11. $^{13}$C NMR of 1 (100 MHz, DMSO-d$_6$).
Fig. S12. HRMS (LC/MS) spectra of 1. The peak at m/z = 438.1705 was assigned to the mass of 1+H^+.

Fig. S13. ^1H NMR of 2 (400 MHz, CDCl₃).
Fig. S14. $^{13}$C NMR of 2 (100 MHz) in CDCl$_3$.

Fig. S15. HRMS (LC/MS) spectra of 2. The peak at m/z = 478.2012 was assigned to the mass of 2+H$^+$. 
Fig. S16. $^1$H NMR of 3 (400 MHz, CDCl$_3$).

Fig. S17. $^{13}$C NMR of 3 (100 MHz) in CDCl$_3$. 
**Fig. S18.** HRMS (LC/MS) spectra of 3. The peak at m/z = 573.2024 was assigned to the mass of 3+H⁺.

**Fig. S19.** ¹H NMR of 4 (400 MHz, CDCl₃).
Fig. S20 $^{13}$C NMR of 4 (100 MHz) in CDCl$_3$

Fig. S21 HRMS (LC/MS) spectra of 4. The peak (m/z) at 668.1336 and the peak (m/z) at 604.1707 corresponded (M + H)$^+$ and (M + H - SO$_2$)$^+$, respectively.
**Fig. S22** $^1$H NMR of 5 (400 MHz, DMSO-$d_6$).

**Fig. S23** $^{13}$C NMR of 5 (100 MHz) in DMSO-$d_6$. 
Fig. S24 HRMS (LC/MS) spectra of 5. The peak (m/z) at 452.1974 corresponded (M + H)^+.