Electronic Supplementary Information

A novel pyrene based dual-multifunctional fluorescence probe for differential sensing of pH and HSO$_3^-$ and its bioimaging in live cells

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1. Cytotoxicity assays in cells

2. Method for determination of the fluorescence quantum yield

3. Figures captions:

Fig. S1. $^1$H NMR spectra of probe in DMSO-d$_6$.

Fig. S2. $^{13}$C NMR spectra of probe in DMSO-d$_6$.

Fig. S3. HRMS spectra of probe.

Fig. S4. HRMS spectra of probe - HSO$_3^-$.

Fig. S5 (a) The ratios of fluorescence intensity of probe (15.0 µM) containing diverse species in CH$_3$CN/ H$_2$O (1/3, V/V) at pH 7.10 and pH 4.00. Conditions: $\lambda_{ex} = 370$ nm, Ex/Em slit = 5/5 nm. (b) Fluorescence responses (455 nm) of probe (15.0 µM) containing diverse species in CH$_3$CN/ H$_2$O (1/19, V/V) at pH 7.10 and 8.50. Conditions: $\lambda_{ex} = 370$ nm, Ex/Em slit = 2.5/5 nm. Ca$^{2+}$ (10 mM); Na$^+$ (150 mM); K$^+$ (150 mM); other metal ions with 0.2 mM, cysteine (1 mM); homocysteine (1 mM); glutathione (1 mM); glycine (1 mM); valine (1 mM); arginine (1 mM); lysine (1 mM); tyrosine (1 mM).

Fig. S6 (a) Time courses of fluorescence emission ratios I$_{460 \text{ nm}}$/I$_{414 \text{ nm}}$ in various pH values (7.10, 4.50 and 2.50, respectively). Conditions: $\lambda_{ex} = 370$ nm, Ex/Em slit = 5/5 nm. (b) Changes in the fluorescence intensity at 455 nm for probe (15.0 µM) in CH$_3$CN/ H$_2$O (1/19, V/V) at pH 7.40, 10.30, 12.30, respectively. Conditions: $\lambda_{ex} = 370$ nm, Ex/Em slit = 2.5/5 nm.

Fig. S7 (a) Reversible changes in the fluorescence emission ratio (I$_{460 \text{ nm}}$/I$_{414 \text{ nm}}$) for probe (15.0 µM) in CH$_3$CN/H$_2$O (1/3, v/v) system between pH 7.10 and 1.36. Conditions: $\lambda_{ex} = 370$ nm, Ex/Em slit = 5/5 nm. (b) Changes in the fluorescence intensity of probe (15.0 µM) in CH$_3$CN/H$_2$O (1/19, v/v) system at
455 nm between pH 7.10 and 13.09. Conditions: $\lambda_{ex} = 370$ nm, Ex/Em slit = 2.5/5 nm.

**Fig. S8** Effect of pH on the fluorescent intensity of probe addition reaction system by bisulfite.

**Fig. S9** Time-dependent fluorescence spectra of probe (15.0 µM) with HSO$_3^-$ (50.0 nM) in PBS buffer (pH = 5.00, 1.5% DMSO). Conditions: $\lambda_{ex} = 420$ nm, Ex/Em slit = 5/10 nm.

**Table S1** Comparison of probe with the reported fluorescence probes for HSO$_3^-$.

**Fig. S10** The fluorescence intensity of probe (15.0 µM) with HSO$_3^-$ (70.0 nM) and other various analytes (100 equiv.) in the PBS buffer (pH = 5.00, 1.5% DMSO). Conditions: $\lambda_{ex} = 420$ nm, Ex/Em slit = 5/10 nm.

**Fig. S11** Fluorescence intensity of probe (15.0 µM) at 555 nm in PBS buffer (pH = 5.00, 1.5% DMSO) to various anions (30 equiv.), and it’s competition graph with bisulfite. Black bar: probe + various species. Red bar: probe + various species + bisulfite. $\lambda_{ex}/\lambda_{em} = 420/555$ nm. Ex/Em slit = 5/10 nm.

**Fig. S12** Partial $^1$H NMR spectrum of probe and probe - OH$^-$ in DMSO-d6.

**Figure S13** (a) Absorbance spectral changes of probe in DMSO, upon increasing the concentration of water (0-100%) (Note: the spectra were taken after 18 hours). (b) Fluorescence spectra of probe in DMSO upon increasing the concentration of water from 0% to 100%. (Note: the spectra were taken after 18 hours).

**Figure S14** (a) Absorbance spectral changes of probe in CH$_3$CN, upon increasing the concentration of water (0-100%) (Note: the spectra were taken after 18 hours). (b) Fluorescence spectra of probe in CH$_3$CN upon increasing the concentration of water from 0% to 100%. (Note: the spectra were taken after 18 hours).

**Table S2** The quantum yield ($\Phi$) of probe in DMSO upon increasing the concentration of water
from 0 % to 100%.

**Table S3** The quantum yield ($\Phi$) of probe in CH$_3$CN upon increasing the concentration of water from 0 % to 100%.

**Fig S15** Cytotoxicity data results obtained from the MTT assay.
1. Cytotoxicity assays in cells

The A549 cells were maintained in a humidified atmosphere containing 5% CO₂ at 37 °C in DMEM supplemented with 100 units of penicillin, 100 μg mL⁻¹ of streptomycin, and 10% fetal bovine serum. The cytotoxicity (IC50) of probe was determined using a MTT assay, a standard method to detect cell survival fraction, by incubating A549 cells. Briefly, the cells with a density of 1×10⁴ cells well⁻¹ were cultured in 96-well glass-bottom plates for 48 h under 5% CO₂. Then the cells were incubated with various concentrations of probe (0, 1, 5, 10, 15, 20, 40, 60, 80, 100 μM) for 12h. At least six parallel samples were created in each group. After that, the suspension medium was removed and 10 μL (5 mg/mL in PBS pH = 7.40) MTT (5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide) was added to each well and performed for 4 h. When the incubation was finished, culture supernatants were aspirated away and purple formazan crystals were dissolved into 150 μL of DMSO for additional incubation of 15 min, China). The cell viability was estimated according to the following equation:

Inhibition rate (IR %) = [OD (control) – OD (drug treated cell)]/ [OD(control)] × 100%

2. Method for determination of the fluorescence quantum yield

For determination fluorescence quantum yields (Φ) of probe, the quinine sulphate in 0.1 M H₂SO₄ solution was used as a fluorescence standard. The fluorescence quantum yields (Φ) were obtained using the following equation:

$$\Phi = \Phi_{\text{ref}} \times \frac{F_{\text{sample}}}{F_{\text{ref}}} \times \frac{A_{\text{ref}}}{A_{\text{sample}}} \times \frac{\eta_{\text{sample}}}{\eta_{\text{ref}}}$$

where sample and ref indicated the unknown and standard solution, respectively. $\Phi = $ quantum at the exaction wavelength, and $\eta = $ refractive index of the solvent. Here $\Phi_{\text{ref}}$ measurements were performed using quinine sulphate in 0.1 M H₂SO₄ as a standard [$\Phi = 0.546$].
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Calculated m/z = 334.12264

Obtained m/z = 334.12323

Calculated m/z = 415.08783

Obtained m/z = 415.08704
Fig. S5 (a) The ratios of fluorescence intensity of probe (15.0 µM) containing diverse species in CH$_3$CN/ H$_2$O (1/3, V/V) at pH 7.10 and pH 4.00. Conditions: $\lambda_{ex} = 370$ nm, Ex/Em slit = 5/5 nm.

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mM); Na$^+$ (150 mM); K$^+$ (150 mM); other metal ions with 0.2 mM, cysteine (1 mM); homocysteine (1 mM); glutathione (1 mM); glycine (1 mM); valine (1 mM); arginine (1 mM); lysine (1 mM); tyrosine (1 mM).
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![Graph](image)

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**Fig. S8** Effect of pH on the fluorescent intensity of probe addition reaction system by bisulfite.
Fig. S9 Time-dependent fluorescence spectra of **probe** (15.0 µM) with HSO$_3^-$ (50.0 nM) in PBS buffer (pH = 5.00, 1.5% DMSO). Conditions: $\lambda_{ex} = 420$ nm, Ex/Em slit = 5/10 nm.
Table S1 Comparison of probe with the reported fluorescence probes for HSO$_3^-$:

<table>
<thead>
<tr>
<th>Probe</th>
<th>Response time</th>
<th>Detection limit</th>
<th>Solution</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Image" /></td>
<td>5 min</td>
<td>100 nM</td>
<td>Water-DMSO (99/1, V/V)</td>
<td>Dyes Pigments, 2017 $^2$</td>
</tr>
<tr>
<td><img src="image2.png" alt="Image" /></td>
<td>a few minutes</td>
<td>28200 nM</td>
<td>Britton-Robinson buffer (20 mM, pH 7)-DMSO (99/1, V/V)</td>
<td>Sens Actuators B:Chemica, 2017 $^3$</td>
</tr>
<tr>
<td><img src="image3.png" alt="Image" /></td>
<td>7.7 min</td>
<td>3060 nM</td>
<td>Sugar (5.0 g per/100 ML)</td>
<td>Talanta, 2017 $^4$</td>
</tr>
<tr>
<td><img src="image4.png" alt="Image" /></td>
<td>20 min</td>
<td>100 nM</td>
<td>HEPES (10 mM, pH 7.40) THF/H$_2$O (1/1, V/V)</td>
<td>Dyes Pigments, 2017 $^5$</td>
</tr>
<tr>
<td>Time</td>
<td>Final Concentration</td>
<td>Buffer</td>
<td>Journal, Year</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>---------------------</td>
<td>-----------------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>300 nM</td>
<td>PBS buffer (20 mM) Containing 1mM CTAB</td>
<td>Ana. Chim. Acta, 2013</td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>1.9 nM</td>
<td>PBS (pH 5, 1.5% DMSO)</td>
<td>This work</td>
<td></td>
</tr>
</tbody>
</table>
**Fig. S10** The fluorescence intensity of **probe** (15.0 µM) with HSO$_3^-$ (70.0 nM) and other various analytes (100 equiv.) in the PBS buffer (pH = 5.00, 1.5% DMSO). Conditions: $\lambda_{ex} = 420$ nm, Ex/Em slit = 5/10 nm.
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Fig. S12 Partial $^1$H NMR spectrum of probe and probe - OH$^-$ in DMSO-d$_6$. 

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Figure S13  (a) Absorbance spectral changes of probe in DMSO, upon increasing the concentration of water (0-100%) (Note: the spectra were taken after 18 hours). (b) Fluorescence spectra of probe in DMSO upon increasing the concentration of water from 0%
to 100%. (Note: the spectra were taken after 18 hours).

Figure S14 (a) Absorbance spectral changes of probe in CH$_2$CN, upon increasing the concentration of water (0-100%) (Note: the spectra were taken after 18 hours). (b)
Fluorescence spectra of probe in CH$_3$CN upon increasing the concentration of water from 0 % to 100%. (Note: the spectra were taken after 18 hours).

**Table S2** The quantum yield (Φ) of probe in DMSO upon increasing the concentration of water from 0 % to 100%.

<table>
<thead>
<tr>
<th>$f_w$</th>
<th>0%</th>
<th>20%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
<th>75%</th>
<th>80%</th>
<th>90%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Φ</td>
<td>0.078</td>
<td>0.060</td>
<td>0.081</td>
<td>0.109</td>
<td>0.173</td>
<td>0.199</td>
<td>0.121</td>
<td>0.065</td>
<td>0.013</td>
</tr>
</tbody>
</table>

**Table S3** The quantum yield (Φ) of probe in CH$_3$CN upon increasing the concentration of water from 0 % to 100%.

<table>
<thead>
<tr>
<th>$f_w$</th>
<th>0%</th>
<th>20%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
<th>75%</th>
<th>80%</th>
<th>90%</th>
<th>100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Φ</td>
<td>0.029</td>
<td>0.050</td>
<td>0.061</td>
<td>0.066</td>
<td>0.090</td>
<td>0.106</td>
<td>0.100</td>
<td>0.054</td>
<td>0.013</td>
</tr>
</tbody>
</table>
Fig. S15 Cytotoxicity data results obtained from the MTT assay.
References


