Supporting Information

A Thiocoumarin-Based Colorimetric and Ratiometric Fluorescent Probe for Hg$^{2+}$ in Aqueous Solution and Its Application in Live-Cell Imaging

Siyao Qin, Bo Chen, Jing Huang, and Yifeng Han*

Department of Chemistry, The Key Laboratory of Advanced Textile Materials and Manufacturing Technology, Zhejiang Sci-Tech University, Hangzhou, 310018, China.
E-mail: zstuchem@gmail.com
**Photophysical properties of MS4**

**Table S1** Photophysical properties of the probe.

<table>
<thead>
<tr>
<th>entry</th>
<th>$\lambda_{ab}$ (nm)</th>
<th>$\lambda_{em}$ (nm)</th>
<th>$\Phi$</th>
<th>$\varepsilon$ / M⁻¹ cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS4</td>
<td>485</td>
<td>543</td>
<td>0.079</td>
<td>33273</td>
</tr>
<tr>
<td>MS4+Hg²⁺</td>
<td>386</td>
<td>477</td>
<td>0.081</td>
<td>28521</td>
</tr>
</tbody>
</table>

(a) The quantum yield ($\Phi$) of MS4 and MS4-Hg²⁺ system were determined according to the literature.¹ (b) $\Phi$ was determined in the present of 2.0 equiv. of Hg²⁺.

$$
\Phi_{\text{Sample}} = \frac{\Phi_{\text{QS}} \cdot A_{\text{QS}} \cdot F_{\text{Sample}} \cdot \lambda_{\text{ex,QS}} \cdot \eta_{\text{Sample}}^2}{A_{\text{Sample}} \cdot F_{\text{QS}} \cdot \lambda_{\text{ex,Sample}} \cdot \eta_{\text{QS}}^2}
$$

Where $\Phi$ is quantum yield; $A$ is absorbance at the excitation wavelength; $F$ is integrated area under the corrected emission spectra; $\lambda_{\text{ex}}$ is the excitation wavelength; $\eta$ is the refractive index of the solution; the Sample and QS refer to the sample and the standard, respectively. We chose Rhodamine 6G in EtOH as standard, which has the quantum yield of 0.95.²
Additional spectroscopic data

**Fig. S1** The UV-vis absorption of MS4 (10.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) at 485 nm and 386 nm as a function of Hg$^{2+}$ concentration (0-3.0 equiv.).
Fig. S2 The ratio of UV-vis absorption of MS4 (10.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) at 485 nm and 386 nm (A<sub>386</sub> nm/A<sub>485</sub> nm and A<sub>485</sub> nm/A<sub>386</sub> nm) as a function of Hg<sup>2+</sup> concentration (0-3.0 equiv.).
Fig. S3 The absorbance at 386 nm of UV-vis absorption of MS4 (10.0 μM) as a function of Hg$^{2+}$ concentration (0-16.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO).
Fig. S4 The ratio of the fluorescent intensity of MS4 (5.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) at 477 nm and 543 nm (I_{477}/I_{543}) as a function of Hg^{2+} concentration (0-10.0 equiv.) (λ_{ex} = 420 nm).
The ratio of the fluorescent intensity of MS4 (5.0 μM) at 477 nm and 543 nm (I_{477}/I_{543}) as a function of Hg^{2+} concentration (0-1.5 μM) under the same condition as the Hg^{2+} titration.

The detection limit (DL) of Hg^{2+} using MS4 was determined from the following equation:  

\text{DL} = 3 \sigma / K

Where \sigma is the standard deviation of the blank solution; K is the slope of the calibration curve.
Fig. S6 Time-dependent Absorption spectra of MS4 (10.0 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) in the presence of 2.0 equiv. of Hg\textsuperscript{2+}. 
**Fig. S7** $^1$H NMR titration experiment of MS4 in the present of Hg$^{2+}$ in $d_6$-DMSO.
**Fig. S8** Fluorescence responses of MS4 (5.0 μM) with various metal ions (including Na⁺, K⁺, Ca²⁺, Mg²⁺, Zn²⁺, Co²⁺, Cr³⁺, Cu²⁺, Fe²⁺, Fe³⁺, Ni²⁺, Pb²⁺, Sn⁴⁺, Ag⁺, and Hg²⁺) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) (λₑₓ = 420 nm).
**Fig. S9** Fluorescence responses of MS4 (5.0 μM) with Ag⁺, and Hg²⁺ in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO and 100 mM NaCl) (λex = 420 nm).
Fig. S10 Fluorescence responses of MS4 (5.0 μM) in the presence of 3.0 equiv. of various metal ions (including Na⁺, K⁺, Ca²⁺, Mg²⁺, Zn²⁺, Co²⁺, Cr³⁺, Cu²⁺, Fe²⁺, Fe³⁺, Ni²⁺, Pb²⁺, Sn⁴⁺, and Ag⁺) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO), followed by 2.0 equiv. of Hg²⁺ (λ<sub>ex</sub> = 420 nm).
Fig. S11 pH-dependent experiment of MS4 (5.0 μM) ($\lambda_{ex} = 420$ nm).
Fig. S12 pH-dependent experiment of MS4 (5.0 μM) in the presence of Hg²⁺ (2.0 equiv.) \((λ_{ex} = 420 \text{ nm})\).
Fig. S13 The experiments between MS4 (5.0 μM) with different source of Hg$^{2+}$ (2.0 equiv. HgCl$_2$, Hg(NO$_3$)$_2$ and Hg(ClO$_4$)$_2$, respectively) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO), ($\lambda_{ex}$ = 420 nm).
**Table S2** Comparison of the properties of MS4 and other mercury sensors.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Solvent</th>
<th>Incubation time</th>
<th>LOD</th>
<th>Imaging in vivo</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="Structure 3" /></td>
<td>THF-H(_2)O 1:9</td>
<td>10 min</td>
<td>0.6 μM</td>
<td>+</td>
<td><em>Anal. Chem.</em>, 2017, 89, 12698–12704</td>
</tr>
<tr>
<td><img src="image4.png" alt="Structure 4" /></td>
<td>PBS-DMSO 99:1</td>
<td>5 min</td>
<td>7.5 nM</td>
<td>+</td>
<td><em>New J. Chem.</em>, 2018, 42, 1181-1186</td>
</tr>
<tr>
<td><img src="image5.png" alt="Structure 5" /></td>
<td>Tris-HCl</td>
<td>30 min</td>
<td>+</td>
<td></td>
<td><em>Org. Biomol. Chem.</em>, 2018, 16, 2388–2392</td>
</tr>
<tr>
<td><img src="image6.png" alt="Structure 6" /></td>
<td>CH(_3)CN-HEPES 3:7</td>
<td>0.17 μM</td>
<td>+</td>
<td></td>
<td><em>Inorg. Chem.</em>, 2016, 55, 12052–12060</td>
</tr>
<tr>
<td><img src="image7.png" alt="Structure 7" /></td>
<td>THF-H(_2)O 2:98</td>
<td>10 μM</td>
<td>-</td>
<td></td>
<td><em>J. Mater. Chem. C</em>, 2018, 6, 773-780</td>
</tr>
<tr>
<td><img src="image8.png" alt="Structure 8" /></td>
<td>PBS-DMSO 99:1</td>
<td>&lt; 5 min</td>
<td>9.23 nM</td>
<td>+</td>
<td>This work</td>
</tr>
</tbody>
</table>
Fig. S14 Cell viability of HeLa cells treated with different concentration of MS4 for different time periods. No cytotoxic effect was observed for the cells incubated with MS4 at 10 μM even for 24 h.
CheckCIF/PLATON report of 3

Bond precision: C-C = 0.0034 A

Cell:
- a = 8.7754(8)
- b = 10.9150(9)
- c = 12.9559(11)
- alpha = 90
- beta = 96.034(9)
- gamma = 90

Temperature: 293 K

Volume:
- Calculated: 1234.09(19)
- Reported: 1234.09(19)

Space group:
- Calculated: P 21/c
- Reported: P 1 21/c 1

Hall group:
- Calculated: -P 2ybc
- Reported: -P 2ybc

Moiety formula:
- Calculated: C13 H15 N O S
- Reported: C13 H15 N O S

Sum formula:
- Calculated: C13 H15 N O S
- Reported: C13 H15 N O S

Mr:
- Calculated: 233.32
- Reported: 233.32

Dx,g cm-3:
- Calculated: 1.256
- Reported: 1.256

Z:
- Calculated: 4
- Reported: 4

Mu (mm-1):
- Calculated: 0.241
- Reported: 0.241

F000:
- Calculated: 496.0
- Reported: 496.0

F000’:
- Calculated: 496.67

h,k,lmax:
- Calculated: 10, 13, 15
- Reported: 10, 13, 15

Nref:
- Calculated: 2261
- Reported: 2256

Tmin, Tmax:
- Calculated: 0.906, 0.930
- Reported: 0.919, 1.000

Correction method: # Reported T Limits: Tmin=0.919 Tmax=1.000
AbsCorr = MULTI-SCAN

Data completeness = 0.998

Theta(max) = 25.350

R(reflections) = 0.0496 (1576)

wR2(reflections) = 0.1296 (2256)

S = 1.045

Npar = 147
Cell culture and imaging

HeLa cells were cultured in DMEM (Invitrogen, Carlsbad, CA), supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO\textsubscript{2} at 37 °C. For imaging experiments, exponentially growing cells were seeded in 24-well plate. Cells were cultured at 37°C in a 5% CO\textsubscript{2} atmosphere for 24 h before they were exposed to reagents.

For labeling, the medium was removed and cells were rinsed three times with PBS. Then HeLa cells were incubated with MS4 (10 μM) in PBS (containing 1% EtOH) at 37 °C for 30 min as control. For Hg\textsuperscript{2+} imaging, another set of HeLa cells was preloaded with MS4 (10 μM) in PBS (containing 1% EtOH) at 37 °C for 30 min, rinsed three times with PBS and further treated with Hg\textsuperscript{2+} (20 μM) in PBS at 37 °C for additional 30 min. Cells were rinsed three times with PBS and bathed in it, then imaging was carried out.
The characterization data of MS4

$^1$H NMR of 2

$^1$H NMR of 3

S21
$^{13}$C NMR of 3

![Carbon NMR spectrum]

MS of 3

![Mass spectrum]

S22
HR-MS of 3

### Qualitative Analysis Report

**Peak List**

<table>
<thead>
<tr>
<th>m/z</th>
<th>Abund</th>
</tr>
</thead>
<tbody>
<tr>
<td>172.0943</td>
<td>237790.6</td>
</tr>
<tr>
<td>234.0959</td>
<td>1985181.9</td>
</tr>
<tr>
<td>235.0978</td>
<td>103323.6</td>
</tr>
<tr>
<td>236.0927</td>
<td>291619.2</td>
</tr>
<tr>
<td>256.0755</td>
<td>100420.0</td>
</tr>
<tr>
<td>485.1661</td>
<td>123727.4</td>
</tr>
<tr>
<td>490.1666</td>
<td>141785.3</td>
</tr>
<tr>
<td>922.0098</td>
<td>110956.7</td>
</tr>
</tbody>
</table>
References

