Supporting Information

A Novel ESIPT-Based Fluorescent Chemodosimeter for Hg$^{2+}$ and Its Application in Live-Cell Imaging

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Photophysical properties of MS3

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

<table>
<thead>
<tr>
<th>entry</th>
<th>$\lambda_{ab}$ (nm)</th>
<th>$\lambda_{em}$ (nm)</th>
<th>$\Phi^a$</th>
<th>$\varepsilon$ / M$^{-1}$ cm$^{-1}$</th>
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<tbody>
<tr>
<td>MS3</td>
<td>438</td>
<td>501</td>
<td>0.01</td>
<td>7520</td>
</tr>
<tr>
<td>MS3+Hg$^{2+}$</td>
<td>386</td>
<td>501</td>
<td>0.49$^b$</td>
<td>7931</td>
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(a) The quantum yield ($\Phi$) of MS3 and MS3-Hg$^{2+}$ system were determined according to the literature.$^1$ (b) $\Phi$ was determined in the present of 15.0 equiv. of Hg$^{2+}$.

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

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 fluorescein in 0.1 M NaOH as standard, which has the quantum yield of 0.95.$^2$
Additional spectroscopic data

**Fig. S1** The ratio of the UV-vis absorption of **MS3** (20.0 μM) at 386 nm and 438 nm as a function of Hg²⁺ concentration in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO).
Fig. S2. The UV-vis absorption of MS3 to various metal ions (5.0 equiv. of each, including Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\), Co\(^{2+}\), Cr\(^{3+}\), Cu\(^{2+}\), Fe\(^{2+}\), Fe\(^{3+}\), Ni\(^{2+}\), Pb\(^{2+}\), Sn\(^{4+}\), Ag\(^+\), and Hg\(^{2+}\)) in PBS buffer solution, 10 mM, pH 7.4, containing 1% DMSO.
Fig. S3. The UV-vis absorption of MS3, MS3+Hg\textsuperscript{2+} system, and compound 3 (control) in PBS buffer solution, 10 mM, pH 7.4, containing 1% DMSO.
Fig. S4 Fluorescent intensity of MS3 (10.0 μM) at 501 nm as a function of Hg$^{2+}$ concentration (0-35.0 equiv.) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO). ($\lambda_{ex} = 392$ nm).
Fig. S5 The fluorescent intensity of MS3 (1.0 μM) at 501 nm as a function of Hg\(^{2+}\) concentration (0-2.5 μM) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO), (\(\lambda_{ex} = 392\) nm).

The detection limit (DL) of Hg\(^{2+}\) using MS3 was determined from the following equation:\(^3\)

\[
DL = 3*\sigma/K
\]

Where \(\sigma\) is the standard deviation of the blank solution; K is the slope of the calibration curve.
Fig. S6 FT IR spectra of MS3 and MS3+Hg^{2+}.
Fig. S7 HR-MS spectra of the isolated product of the MS3–Hg2+ solution. HR-MS (TOF-ESI): Calcd. for ([M+H])+, 219.1134; Found, 219.1129.
**Fig. S8** Time-dependent fluorescence intensity changes of MS3 (10.0 μM) alone in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) ($\lambda_{ex} = 392$ nm).
Fig. S9 Time-dependent fluorescence intensity changes of MS3 (10.0 μM) upon addition of 5.0 equiv. of Hg$^{2+}$ in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) ($\lambda_{ex}$ = 392 nm).
Fig. S10 Time-dependent fluorescence intensity changes of MS3 (10.0 μM) upon addition of 10.0 equiv. of Hg$^{2+}$ in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) ($\lambda_{ex} = 392$ nm).
**Fig. S11** Time-dependent fluorescence intensity changes of MS3 (10.0 μM) upon addition of 15.0 equiv. of Hg$^{2+}$ in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) ($\lambda_{ex} = 392$ nm).
Fig. S12 Time-dependent fluorescence intensity changes of MS3 (10.0 μM) upon addition of 20.0 equiv. of Hg$^{2+}$ in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) ($\lambda_{ex} = 392$ nm).
Fig. S13 The pseudo-first-order kinetic plot of the reaction of the probe MS3 (10.0 μM) with Hg$^{2+}$ (150.0 μM) at 501 nm in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO) ($\lambda_{ex} = 392$ nm). Slope = 0.612 min$^{-1}$;
**Fig. S14** Fluorescence responses of MS3 (10.0 μM) to various metal ions (15.0 equiv. of each metal ions, including Na⁺, K⁺, Ca²⁺, Mg²⁺, Mn²⁺, Zn²⁺, Co²⁺, Cr³⁺, Cu²⁺, Fe²⁺, Fe³⁺, Ni²⁺, Pb²⁺, Sn⁴⁺, Ag⁺, and Hg²⁺) in PBS buffer solution, 10 mM, pH 7.4, containing 1% DMSO (λ<sub>ex</sub> = 392 nm).
Fig. S15 Fluorescence responses of MS3 (10.0 μM) in the presence of 15.0 equiv. of metal ions (including Na\(^+\), K\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), Mn\(^{2+}\), Zn\(^{2+}\), Co\(^{2+}\), Cr\(^{3+}\), Cu\(^{2+}\), Fe\(^{2+}\), Fe\(^{3+}\), Ni\(^{2+}\), Pb\(^{2+}\), Sn\(^{4+}\), Ag\(^+\), and Hg\(^{2+}\)) in PBS buffer solution (10 mM, pH 7.4, containing 1% DMSO), followed by 15.0 equiv. of Hg\(^{2+}\) (λ\(_{ex}\) = 392 nm).
Fig. S16 Effect of the pH on the fluorescence intensity at 501 nm of MS3 (10.0 μM) alone and in the presence of Hg$^{2+}$ (15.0 equiv.) ($\lambda_{ex} = 392$ nm).
**Fig. S17** Effect of the pH on the fluorescence intensity at 501 nm of MS3 (10.0 μM) alone ($\lambda_{ex} = 392$ nm).
**Fig. S18** Effect of the pH on the fluorescence intensity at 501 nm of MS3 (10.0 μM) in the presence of Hg$^{2+}$ (15.0 equiv.) ($\lambda_{ex} = 392$ nm).
Fig. S19 HOMO and LUMO energy levels of the frontier molecular orbitals of MS3 (probe) and compound 3 (3-aminophthalimide).
Cell lines and imaging experiments

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 MS3 (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 MS3 (10 μM) in PBS (containing 1% EtOH) at 37 °C for 30 min, rinsed three times with PBS and further treated with different concentrations of Hg\textsuperscript{2+} (10, 15, and 20 μM, respectively) 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. Images were acquired using an inverted fluorescence microscope and fluorescence imaging was performed in a blue channel.
Fig. S20 Cell viability of HeLa cells treated with different concentration of MS3 for different time periods. No cytotoxic effect was observed for the cells incubated with MS3 at 10 μM even for 24 h.
The characterization data of MS3

$^1$H NMR of 2

$^1$H NMR of 3
$^{13}$C NMR of 4 (MS3)

2D NMR (HMBC) of 4 (MS3)
Partial enlarge of HMBC spectrum of 4 (MS3)

HRMS of 4 (MS3)

Qualitative Analysis Report

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References

