Supporting Information

A Rhodamine/BODIPY-Based Fluorescent Probe for the Differential Detection of Hg(II) and Au(III)†

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† Dedicated to the memory of Prof. Dr. Ayhan S. Demir (1950-2012)

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1. **General Methods:** All reagents were purchased from commercial suppliers (Aldrich and Merck) and used without further purification. $^1$H NMR and $^{13}$C NMR were measured on a Varian VNMRJ 400 Nuclear Magnetic Resonance Spectrometer. Bruker MALDI-TOF-TOF Mass Spectrometer was used for mass spectrometry analysis. UV absorption spectra were obtained on Shimadzu UV-2550 Spectrophotometer. Fluorescence emission spectra were obtained using Varian Cary Eclipse Fluorescence spectrophotometer. Cell imaging was performed with Olympus CKX41 fluorescence microscope. Samples were contained in 10.0 mm path length quartz cuvettes (2.0 mL volume). Upon excitation at 470 nm, the emission spectra were integrated over the range 480 nm to 700 nm for Au$^{3+}$ measurements. Upon excitation at 525 nm, the emission spectra were integrated over the range 535 nm to 700 nm for Hg$^{2+}$ measurements. The pH was recorded by HI-8014 instrument (HANNA). All measurements were conducted at least in triplicate.

2. **Synthesis Section**

**Synthesis of RhS-BOD**

Scheme S1 Synthesis route of RhS-BOD
Compound 1 was synthesized according to known procedure. Formylation reaction of compound 1 was performed by using known (Vilsmeier Haack reaction) procedure. To a solution of rhodamine B (150mg, 0.33 mmol) in absolute ethanol (10 ml) was added BODIPY-AL (116 mg, 0.33 mmol) and the solution was stirred overnight at reflux temperature. The reaction mixture was extracted with dichloromethane (3 x 10 mL). Then, the collected organic layers were dried over anhydrous MgSO₄, concentrated under vacuum, and purified by column chromatography (hexane/EtOAc = 8/1) to give 156 mg of RhO-BOD (60%) as a pink oil. H-NMR (400 MHz, CDCl₃) δ: 9.12 (s, 1H), 7.94-7.92 (m, 1H), 7.49-7.45 (m, 4H), 7.20-7.12 (m, 3H), 6.98 (t, J=7.2 Hz, 1H), 6.47-6.26 (m, 6H), 5.99 (s, 1H), 3.32 (br. s, 8H), 2.47 (s, 3H), 2.31 (s, 3H), 1.33 (s, 6H), 1.15 (br. s, 12H). 13C NMR (100 MHz, CDCl₃) δ: 164.13, 156.58, 153.37, 150.80, 148.80, 143.88, 142.01, 140.88, 137.76, 134.76, 132.06, 131.99, 130.92, 130.61, 129.88, 129.18, 128.28, 127.89, 126.00, 124.04, 123.06, 121.89, 107.88, 106.28, 97.75, 66.23, 44.36, 14.68, 14.47, 14.16, 12.58, 11.99. MS (MALDI-TOF): m/z: Calcd. for C₄₈H₄₉BF₂N₆O₂: 791.479 [M+H]+, Found: 791.471 [M+H]+.

The RhO-BOD (100 mg, 0.13 mmol) and Lawesson's reagent (53 mg, 0.13 mmol) were dissolved in dry toluene, and reaction mixture was refluxed for 2 h under N₂ atmosphere. After removal of toluene, the residue was purified by column chromatography (hexane/EtOAc = 10/1) to give 42 mg of RhS-BOD (40%) as a purple solid. H-NMR (400 MHz, CDCl₃) δ: 8.63 (s, 1H), 8.07 (dd, J=6.0 Hz, 3.2 Hz, 1H), 7.48-7.47 (m, 3H), 7.39 (dd, J=6.0 Hz, 3.2 Hz, 2H), 7.27-7.26 (m, 2H), 7.10 (dd, J=6.0 Hz, 3.2 Hz, 1H), 6.76 (d, J=8.4 Hz, 2H), 6.30-6.27 (m, 4H), 6.04 (s, 1H), 3.32 (q, J=7.2 Hz, 8H), 2.81 (s, 3H), 2.58 (s, 3H), 1.59 (s, 3H), 1.38 (s, 3H), 1.15 (t, J=7.2 Hz, 12H). 13C NMR (100 MHz, CDCl₃) δ: 170.92, 157.99, 156.58, 155.55, 152.94, 151.74, 148.14, 144.95, 142.49, 141.94, 135.05, 134.71, 131.96, 130.36, 129.24, 129.16, 127.92, 127.70, 127.12, 123.93, 122.48, 122.09, 110.58, 108.17, 97.38, 62.68, 44.34, 14.81, 14.64, 14.09, 12.88, 12.64. MS (MALDI-TOF): m/z: Calcd. for C₄₈H₄₉BF₂N₆O₅S: 807.375 [M+H]+, Found: 807.451 [M+H]+.

### 3. Cell Imaging

Human A549 lung adenocarcinoma cell lines were grown in DMEM supplemented with 10% FBS (fetal bovine serum) in an atmosphere of 5% CO₂ at 37 °C. The cells were plated on 12mm cover glasses in 6-well plate and allowed to grow for 24 h. Before the experiments, the cells were washed with PBS buffer, and then the cells were incubated RhS-BOD (5 µM) for 40 min at 37 °C then washed with PBS three times. After incubating with Au³⁺ (5 µM) or Hg²⁺ (5 µM) for 20 min at 37 °C, cells were rinsed with PBS three times, and DAPI for 15
min at 37 °C then washed with PBS three times. Then the fluorescence images were acquired through an Olympus CKX41 fluorescence microscope.

**References**


4. Absorption and Emission Spectra of RhS-BOD with Hg$^{2+}$

![Absorption Spectra](image1)

![Fluorescence Intensity](image2)

**Figure S1** Absorption and Emission spectra of RhS-BOD (5 µM) and Hg$^{2+}$ (1.0 equiv.) in 1:1 CH$_3$CN/HEPES buffer at pH = 7.0; ($\lambda_{ex}$: 525 nm).
5. Time-dependent Fluorescence Change of RhS-BOD with Hg$^{2+}$

![Fluorescence Intensity vs Wavelength graph]

**Figure S2** Time-dependent fluorescence change of **RhS-BOD** (5 µM) in the presence of an 1.0 equivalent of HgCl$_2$ measured in 1:1 CH$_3$CN/HEPES buffer at pH = 7.0
6. Fluorescence Titration of RhS-BOD with Hg$^{2+}$

**Figure S3** Fluorescence spectra of RhS-BOD (5 µM) in 1:1 CH$_3$CN/HEPES buffer at pH = 7.0 in the presence of Hg$^{2+}$ (mole equivalents = 0.01 - 5.0)

**Figure S4** Fluorescence intensity changes of RhS-BOD ($\lambda_{max}$: 585 nm) vs equivalents of Hg$^{2+}$
7. The Fluorescence Responses of RhS-BOD with Hg$^{2+}$ and Other Metals

**Figure S5** Fluorescence intensities of RhS-BOD (5 µM) in 1:1 CH$_3$CN/HEPES buffer at pH = 7.0 at $\lambda_{\text{max}}$: 585 nm in the presence of 10.0 equivalent of the cations interest: 1, RhS-BOD only; 2, Hg$^{2+}$ (1.0 equiv.); 3, Cu$^{2+}$; 4, Ag$^+; 5$, Zn$^{2+}; 6$, Pb$^{2+}; 7$, Ni$^{2+}; 8$, Na$^+$; 9, Mg$^{2+}; 10$, Li$^+$; 11, K$^+$; 12, Pd$^{2+}; 13$, Fe$^{3+}; 14$, Co$^{2+}; 15$, Cd$^{2+}; 16$, Ca$^{2+}; 17$, Ba$^{2+}; 18$, Fe$^{5+}; 19$, Cr$^{3+}$
8. The Fluorescence Responses of RhS-BOD in the Presence of Hg²⁺ and Other Metal Ions.

**Figure S6** Fluorescence intensities of RhS-BOD (5 µM) in 1:1 CH₃CN/HEPES buffer at pH = 7.0 at λ_{max}: 585 nm in the presence of Hg²⁺ (1.0 equiv.) and 10.0 equiv of the following metal ions: 1, none; 2, Cu²⁺; 3, Ag⁺; 4, Zn²⁺; 5, Pb²⁺; 6, Ni²⁺; 7, Na⁺; 8, Mg²⁺; 9, Li⁺; 10, K⁺; 11, Pd²⁺; 12, Fe²⁺; 13, Co²⁺; 14, Cd²⁺; 15, Ca²⁺; 16, Ba²⁺; 17, Cr³⁺; 18, Fe³⁺.

9. The Fluorescence Intensity Changes of RhS-BOD in the Presence of Hg²⁺ and CN⁻ Ions.

**Figure S7** Fluorescence intensity changes of RhS-BOD (5 µM) in 1:1 CH₃CN/HEPES buffer at pH = 7.0 at λ_{max}: 585 after addition of 1 equiv. Hg²⁺, 1 equiv. Hg²⁺ + 10 equiv. CN⁻ and 1 equiv. Hg²⁺ + 10 equiv. CN⁻ + 10 equiv. Hg²⁺ respectively.
10. Effect of pH

Figure S8 Effect of pH on the fluorescence intensity of RhS-BOD (5 µM) in 1:1 CH₃CN/HEPES in the absence (a) and presence (b) of Hg²⁺ (1.0 equiv.) $\lambda_{\text{max}}$: 585

11. Job's Plot

Figure S9 Job’s plots for the RhS-BOD and Hg²⁺ in 1:1 CH₃CN/HEPES solution
12. Determination of Detection Limit of Hg$^{2+}$

The detection limit was calculated based on the fluorescence titration. To determine the S/N ratio, the emission intensity of RhS-BOD (5 µM) without Hg$^{2+}$ was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and Hg$^{2+}$ concentration could be obtained in the 0 - 0.3 µM (R = 0.9885). The detection limit is then calculated with the equation: detection limit = 3σbi/m, where σbi is the standard deviation of blank measurements; m is the slope between intensity versus sample concentration. The detection limit was measured to be 8 nM at S/N = 3.

![Graph showing fluorescence changes and spectra](Figure S10(a) and Figure S10(b))

**Figure S10 (a)** Fluorescence changes of RhS-BOD (5.0 µM) upon addition of Hg$^{2+}$ (0.05 to 0.3 µM, 0.01 to 0.05 equiv.) **(b)** Fluorescence spectra of RhS-BOD (5.0 µM) in the presence of Hg$^{2+}$ (0.05 µM, 0.01 equiv.) in 1:1 CH$_3$CN/HEPES buffer at pH = 7.0
13. Absorption and Emission Spectra of RhS-BOD with Au$^{3+}$ at $\lambda_{ex}$: 470 nm

\begin{figure}
\centering
\includegraphics[width=\textwidth]{absorption_spectrum.png}
\caption{Absorption and Emission spectra of RhS-BOD (5 µM) and Au$^{3+}$ (1.0 equiv.) in 1:1 CH$_3$CN/HEPES buffer at pH = 7.0}
\end{figure}
14. Time-dependent Fluorescence Change of RhS-BOD with $\text{Au}^{3+}$ at $\lambda_{\text{ex}}$: 470 nm

**Figure S12** Time-dependent fluorescence change of **RhS-BOD** (5 µM) in the presence of an 1.0 equivalent of AuCl$_3$ measured in 1:1 CH$_3$CN/HEPES buffer at pH = 7.0
15. Fluorescence Titration of RhS-BOD with Au^{3+} at $\lambda_{ex}$: 470 nm and $\lambda_{ex}$: 525 nm

![Fluorescence spectra of RhS-BOD (5 µM) in 1:1 CH$_3$CN/HEPES buffer at pH = 7.0 in the presence of Au$^{3+}$ (mole equivalents = 0 - 10.0) (a) $\lambda_{ex}$: 470 nm (b) $\lambda_{ex}$: 525 nm](#)

**Figure S13** Fluorescence spectra of RhS-BOD (5 µM) in 1:1 CH$_3$CN/HEPES buffer at pH = 7.0 in the presence of Au$^{3+}$ (mole equivalents = 0 - 10.0) (a) $\lambda_{ex}$: 470 nm (b) $\lambda_{ex}$: 525 nm

![Fluorescence intensity changes of RhS-BOD vs equivalents of Au$^{3+}$ (a) $\lambda_{ex}$: 470 nm (b) $\lambda_{ex}$: 525 nm](#)

**Figure S14** Fluorescence intensity changes of RhS-BOD vs equivalents of Au$^{3+}$ (a) $\lambda_{ex}$: 470 nm (b) $\lambda_{ex}$: 525 nm
16. The Fluorescence Responses of RhS-BOD with $\text{Au}^{3+}$ and Other Metals at $\lambda_{\text{ex}}$: 470 nm

![Graph showing fluorescence responses of RhS-BOD with different metal ions.](image)

**Figure S15** Fluorescence intensities of RhS-BOD (5 µM) in 1:1 CH$_3$CN/HEPES buffer at pH = 7.0 at $\lambda_{\text{max}}$: 506 nm in the presence of 10.0 equivalent of the cations interest: 1, RhS-BOD only; 2, Au$^{3+}$ (1.0 equiv.); 3, Au$^{+}$; 4, Cu$^{2+}$; 5, Zn$^{2+}$; 6, Pb$^{2+}$; 7, Ni$^{2+}$; 8, Na$^{+}$; 9, Mg$^{2+}$; 10, Li$^{+}$; 11, K$^+$; 12, Pd$^{2+}$; 13, Fe$^{3+}$; 14, Co$^{2+}$; 15, Cd$^{2+}$; 16, Ca$^{2+}$; 17, Ba$^{2+}$; 18, Ag$^{+}$; 19, Fe$^{3+}$; 20, Cr$^{3+}$.
17. The Fluorescence Responses of RhS-BOD in the Presence of Au$^{3+}$ and Other Metal Ions at $\lambda_{ex}$: 470 nm

![Figure S16](image1)

**Figure S16** Fluorescence intensities of RhS-BOD (5 µM) in 1:1 CH$_3$CN/HEPES buffer at pH = 7.0 at $\lambda_{max}$: 506 nm in the presence Au$^{3+}$ (1.0 equiv.) and 10.0 equiv the following metal ions: 1, none; 2, Au$^+$; 3, Cu$^{2+}$; 4, Zn$^{2+}$; 5, Pb$^{2+}$; 6, Ni$^{2+}$; 7, Na$^+$; 8, Mg$^{2+}$; 9, Li$^+$; 10, K$^+$; 11, Pd$^{2+}$; 12, Fe$^{2+}$; 13, Co$^{2+}$; 14, Cd$^{2+}$; 15, Ca$^{2+}$; 16, Ba$^{2+}$; 17, Ag$^+$; 18, Cr$^{3+}$; 19, Fe$^{3+}$

18. Effect of pH at $\lambda_{ex}$: 470 nm

![Figure S17](image2)

**Figure S17** Effect of pH on the fluorescence intensity of RhS-BOD (5 µM) in 1:1 CH$_3$CN/HEPES in the absence (a) and presence (b) of Au$^{3+}$ (1.0 equiv.) $\lambda_{max}$: 506 nm
19. Determination of Detection Limit of Au$^{3+}$ at $\lambda_{ex}$: 470 nm

The detection limit was calculated based on procedure mentioned before and the detection limit was measured to be 65 nM at S/N = 3.

![Graph showing fluorescence changes and spectra](image)

**Figure S18 (a)** Fluorescence changes of RhS-BOD (5.0 μM) upon addition of Au$^{3+}$ (0.05 to 0.3 μM, 0.01 to 0.05 equiv.) **(b)** Fluorescence spectra of RhS-BOD (5.0 μM) in the presence of Au$^{3+}$ (0.05 μM, 0.01 equiv.) in 1:1 CH$_3$CN/HEPES buffer at pH = 7.0
20. Determination of detection Limit of Au\(^{3+}\) at \(\lambda_{\text{ex}}: 525\) nm

The detection limit was calculated based on procedure mentioned before and the detection limit was measured to be 10 nM at S/N = 3.

![Graph showing fluorescence changes and spectra](image)

**Figure S19(a)** Fluorescence changes of RhS-BOD (5.0 μM) upon addition of Au\(^{3+}\) (0.05 to 0.3 μM, 0.01 to 0.05 equiv.) at \(\lambda_{\text{ex}}: 525\) nm  **(b)** Fluorescence spectra of RhS-BOD (5.0 μM) in the presence of Au\(^{3+}\) (0.05 μM, 0.01 equiv.) in 1:1 CH\(_3\)CN/HEPES buffer at pH = 7.0
21. Job’s Plot for Au$^{3+}$ at $\lambda_{\text{ex}}$: 525 nm

**Figure S20** Job’s plot for the RhS-BOD and Au$^{3+}$ in 1:1 CH$_3$CN/HEPES solution at $\lambda_{\text{ex}}$: 525 nm
**$\text{H NMR of RhO-BOD}$**

![H NMR spectrum of RhO-BOD](image)

**$\text{C NMR of RhO-BOD}$**

![C NMR spectrum of RhO-BOD](image)
$^1$H NMR of RhS-BOD

$^{13}$C NMR of RhS-BOD

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MALDI-TOF result of **RhO-BOD**

MALDI-TOF result of **RhS-BOD**
22. TLC Image of the Hydrolysis Reaction of RhS-BOD Mediated by Au(III) Ions

![Chemical structure of RhS-BOD and Au(III) mediated reaction to form BODIPY-AL]

**Figure S21** TLC image of the hydrolysis reaction of RhS-BOD mediated with Au (III) ion
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