Electronic Supporting Information

for

A Rhodamine-based Chemosensor for Cu$^{2+}$ and Its Application in Bioimaging

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1. Instruments, reagents and experimental procedures

$^1$H and $^13$C NMR spectra were taken on a Varian mercury-400 spectrometer with TMS as an internal standard and CDCl$_3$ as solvent. Absorption spectra were determined on a Varian UV-Cary100 spectrophotometer. Fluorescence spectra measurements were performed on a Hitachi F-4500 spectrofluorimeter. All pH measurements were made with a pH-10C digital pH meter. HRMS were determined on a Bruker Daltonics APEXII 47e FT-ICR spectrometer.

All the materials for synthesis were purchased from commercial suppliers and used without further purification. Methanol for spectra detection was HPLC reagent without fluorescent impurity.
**Procedures of metal ion sensing**

Stock solutions of the metal ions (2.5 mM) were prepared in deionized water. A stock solution of L1 (1 mM) was prepared in DMF: CH₃CN (1:1 v/v). The solution of L1 was then diluted to 20 μM with water/ CH₃CN (1:1 v/v). In titration experiments, each time a 2 mL solution of I (20μM) was filled in a quartz optical cell of 1 cm optical path length, and the Cu²⁺ stock solution was added into the quartz optical cell gradually by using a micro-pipet. Spectral data were recorded at 2 min after the addition. In selectivity experiments, the test samples were prepared by placing appropriate amounts of metal ion stock into 2 mL solution of L1 (20μM). For fluorescence measurements, excitation was provided at 495nm, and emission was collected from 508 to 650 nm.

**Cell Culture**

The EJ cell line was provided by Institute of Biochemistry and Cell Biology (China). Cells were grown in H-DMEM (Dulbecco’s Modified Eagle’s Medium, High Glucose) supplemented with 10 % FBS (Fetal Bovine Serum) in an atmosphere of 5 % CO₂, 95 % air at 37°C. Cells (5×10⁸/L) were plated on 18 mm glass coverslips and allowed to adhere for 24 hours. Experiments to assess Cu²⁺ uptake were performed in the same media supplemented with 40 μM CuCl₂ for 0.5h.

**Fluorescence Imaging**

Fluorescent pictures were taken on Zeiss Leica inverted epifluorescence/reflectance laser scanning confocal microscope. Excitation of 1-loaded cells at 515 nm was carried out with a HeNe laser. Emission was collected using a 560 nm long-pass filter. Emission was collected from 570 to 625 nm. Before the experiments, cells were washed with PBS buffer and then incubated with 20 μM L1 in DMF-PBS (1:49, v/v) for 5h at 37 °C. Cell imaging was then carried out after washing cells with PBS.
Binding Constant
The binding constant was calculated from the emission intensity - titration curves according to the equation
\[
\frac{I_F^0}{(I_F-I_F^0)} = \left(\frac{1}{f}\right) \left[\frac{1}{K_S[M]}+1\right],
\]
where \(I_F^0\) is the emission intensity of \(L1\) at 552 nm, \(I_F\) is the emission intensity of \(L1\) at 552 nm upon the addition of different amount of \(Cu^{2+}\), \(f\) is the fraction of the initial fluorescence which is accessible to the sensor, \([M]\) is the concentration of \(Cu^{2+}\). The association constant values \(K_S\) is given by the ratio intercept / slope.

The binding constant was also calculated from the absorption intensity - titration curves according to the equation
\[
A = A_0 + \left(\frac{A_{lim} - A_0}{2C_0}\right)(C_0 + [M] + 1/K - ((C_0 + [M] + 1/K)^2 - 4C_0[M])^{0.5}),
\]
where \(A\) and \(A_0\) are the absorbance for \(L1\) (at 529 nm) in the presence and absence of \(Cu^{2+}\), \(C_0\) is half of the concentration of \(L1\); \([M]\) is the concentration of the \(Cu^{2+}\); and \(A_{lim}\) is the limiting value of the absorbance in the presence of excess \(Cu^{2+}\).

2. Synthesis routes and characteristic data

Rhodamine 6G hydrazone \(2\) is prepared according to the literature method.\(^1\)

Rhodamine 6G hydrozide (1.0 mmol, 0.428 g) and furan-2-carbaldehyde (1.0 mmol, 0.096 g) were mixed in boiling ethanol with 3 drops of acetic acid. After 4 h of stirring, brown precipitates obtained were filtered off, washed with ethanol/ether (1:1)
and dried over P₂O₅ under vacuum. Yield: 55 %. ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 8.06-8.027 (m, 2H), 7.434-7.455 (m, 2H), 7.362-7.365 (d, 1H J = 1.2 Hz), 7.261 (s, 2H), 6.366-6.393 (ss, 4H), 3.491 (b, 3H), 3.181-3.235 (q, 4H, J=7.2), 1.873 (s, 6H), 1.299-1.334 (t, 6H, J = 6.8 Hz). ¹³C NMR (CDCl₃, 100 MHz) δ (ppm): 165.14, 150.98, 150.47, 147.60, 143.77, 135.31, 133.49, 128.16, 127.49, 123.50, 123.45, 118.51, 112.04, 111.52, 105.84, 96.90, 65.47, 38.35, 16.66, 14.73. ESI-MS m/z = 507.2 [M + H]⁺, calc. for C₂₇H₂₉N₅O₂S = 506.23.

3. Supplementary spectra data

![Absorption spectra](image)

**Fig. S1** Changes in the absorption spectra of L1 (20 μM) in the presence of different metal ions (40 μM) in water/CH₃CN (1:1 v/v).
Fig.S2 (a) Photos of color changes of L1 (20 μM) upon addition of 40 μM different metal ions in water/ CH₃CN (1:1 v/v) solutions.
Figure S3: Fitting of Fluorescence titration curve of L1 in water/CH₃CN (1:1 v/v) solution.

Equation: $Y = 0.98907 \times 10^{-6} \times X + 0.00881$

$R = 0.99651$
Fig. S4 Fluorescence spectra of L1 (20μM) upon the addition of various metal ions (20μm for Cu$^{2+}$; 40μm for all other cations) in water/CH$_3$CN (1:1 v/v) solution.
Fig. S5. Fluorescence spectra of 20 μM L1 and 30 μM Cu²⁺ upon the addition of 80 μM EDTA in water/CH₃CN (1:1 v/v) solution (λₑₓ = 495 nm).

Reversible binding nature of Cu²⁺ with L1:
**Fig. S6** Fluorescence spectra of 1 (20 μM) with the addition of Cu$^{2+}$ (30 μM) in water/CH$_3$CN (1:1 v/v) at various pH values (Ex. 495 nm; Em. 552 nm).
**Fig. S7** Time course of the response of L1 (20 μM) to 5 equiv Cu$^{2+}$ in water/CH$_3$CN (1:1 v/v) solution.
Figure S8 $^1$H NMR (CDCl$_3$, 400 MHz) spectrum of L1.
**Fig. S9** $^{13}$C NMR (CDCl$_3$, 100MHz) spectrum of L1.
Fig. S10 ESI mass spectrum of L1.
Fig.S11 ESI mass spectrum of 20 μM L1 in the presence of 1.2 equiv of Cu$^{2+}$ in water/CH$_3$CN (1:1 v/v) solution, indicating the formation of [L1+Cu$^{2+}$-H].