Supporting Information

for

A highly sensitive ratiometric fluorescent probe for Cd^{2+} in aqueous solution and living cells

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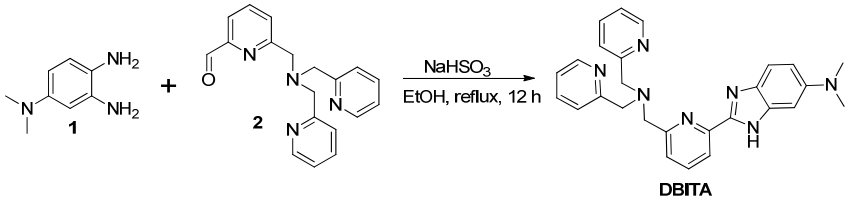
S1. Materials and general methods

All the solvents were of analytic grade. The stock solutions of metal ions for fluorescence discrimination were prepared from $MnCl_2$, $PbCl_2$, $CoCl_2 \cdot 6H_2O$, Zn $(NO_3)_2 \cdot 7H_2O$, $CaCl_2$, NaCl, $CuSO_4$, $NiCl_2 \cdot 6H_2O$, KCl, $FeCl_2$, $CdCl_2 \cdot 2.5H_2O$, $HgCl_2$, $MgCl_2 \cdot 6H_2O$ using doubly distilled water. The ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DRX-500 spectrometer with TMS as internal standard in $CDCl_3$. Mass spectrometric data were determined with a Bruker Autoflex II MALDI-TOF mass spectrometer. Fluorescence measurements were performed on an AMINCO Bowman series 2 with 3 nm slit for both excitation and emission. Absorption spectra were measured on a Shimadzu UV-3100 or an UV-VIS-NIR spectrophotometer. All pH measurements were determined by a Model PHS-3C meter.

S2. Synthesis of DBITA



Scheme 1

Synthesis of DBITA

Compounds **1** (4-*N,N'*-dimethylamino-phenylenediamine) and **2** ([6-formyl(2-pyridyl-methyl)amine]bis(2-pyridylmethyl)amine) were prepared respectively according to the reported procedures.[1,2] For the preparation of **DBITA**, compound **2** (152 mg, 0.46 mmol) dissolved in ethanol (10 mL) was added slowly at room temperature to the ethanol suspension (5 mL) containing compound **1** (70 mg, 0.46 mmol) and $NaHSO_3$ (53 mg, 0.51 mmol) with stirring. Then, the resulting mixture was refluxed with stirring for 12 h. After the mixture was cooled to room temperature, the solvent was removed via evaporation *in vacuo*. The residue was purified by column chromatography (silica gel, EtOAc: methanol = 20: 1, R_f = 0.3), and pure **DBITA** was obtained as a orange oil in 26 % yield. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 3.04 (*s*, 6H, -N(CH_3)$_2$), 3.91 (*s*, 2H, -CH_2), 4.03 (*s*, 4H, -CH_2), 6.93 (*s*, 1H, BI-H), 7.23 (*t*, 2H, *J* = 6.0, Py-H), 7.29 (*d*, 2H, *J* = 7.5, Py-H), 7.66-7.78 (*m*, 6H, BI-H and Py-H), 8.20(*d*, 1H, *J* = 7.5, BI-H), 8.65 (d, 2H, *J* = 4.5, Py-H). ^{13}C NMR (125 MHz, $CDCl_3$, δ, ppm): 41.56, 58.23, 59.90, 93.99, 111.16, 118.95, 119.22, 119.79, 122.17, 122.99, 123.43, 135.77, 136.52, 137.17, 148.09, 148.57, 148.74, 149.69, 156.93, 158.98. MALDI-TOF MS: Calcd. 450.23, found: 450.4 for $[M+H]^+$. Element analysis (%) Calcd. for $C_{27}H_{27}N_7$: C, 72.14; H, 6.05; N, 21.81. Found: C, 72.89; H, 6.54; N, 21.03.

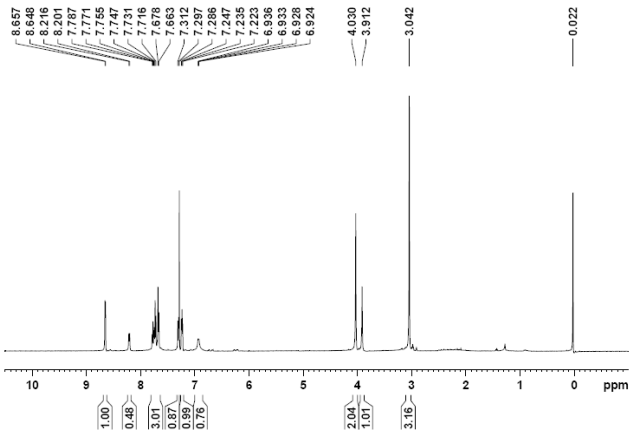


Figure S1. ^{1}H NMR spectrum of **DBITA** in $CDCl_3$.

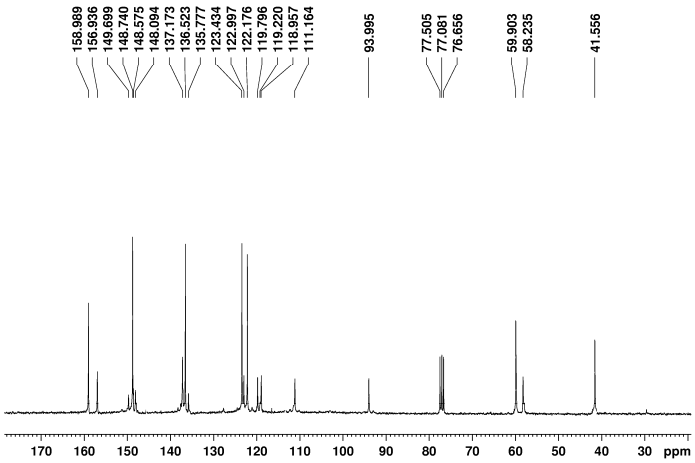


Figure S2. ^{13}C NMR spectrum of **DBITA** in $CDCl_3$.

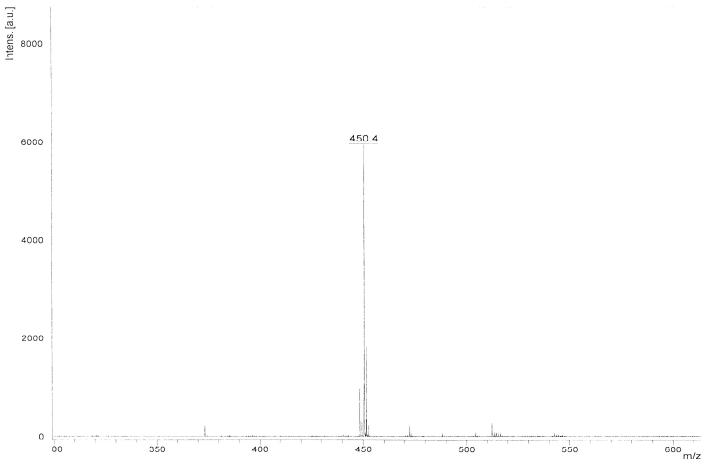


Figure S3. MALDI-TOF spectrum of **DBITA**.

S3. Fluorescence spectra of DBITA

The emission and excitation spectra of **DBITA** (10 μM) were determined in a HEPES buffer (50 mM HEPES, 100 mM KNO_3; pH, 7.2; DMSO/H_2O: 1:9, v/v) using AMINCO Bowman series 2. All UV-vis spectra were recorded by a Shimadzu UV-3100 spectrophotometer and the UV titration experiment of **DBITA** were carried out by adding aliquots of 2.5 μL of $CdCl_2$ aqueous

solution (1.2 mM) to 3 mL of sample solution (10 μM, 50 mM HEPES, 100 mM KNO_3; pH, 7.2; DMSO/H_2O: 1:9, v/v) in a cuvette. The spectra were recorded after the solution was completely mixed. The fluorescence titration experiment was investigated in a similar procedure.

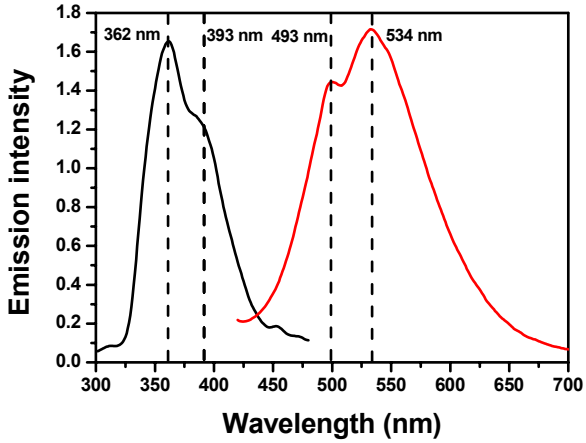


Figure S4. Excitation and emission spectra of **DBITA** (1×10^{-5} M) in HEPES solution.

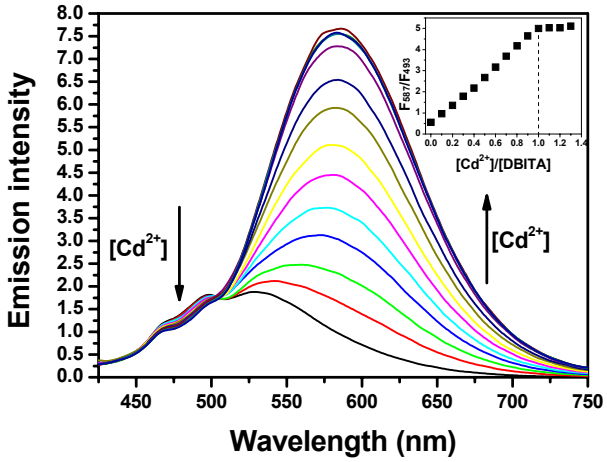


Figure S5. Emission spectra of **DBITA** (1×10^{-5} M) in HEPES buffer obtained by adding aliquots of 12.5 μL $CdCl_2$ (1.2 mM) solution when excited at 405 nm. The $[Cd^{2+}]_{total}$ values are 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 10.0, 11.0, 12.0, 13.0, 14.0 ,15.0 μM (along the direction of arrow). Inset, the titration profile based on the emission ratio at 587 and 493 nm, F_{587}/F_{493}.

S4. Determination of the dissociation constant of Cd^{2+}/DBITA complex.

A series of HEPES buffer (50 mM, pH 7.20, 0.1 M KNO_3) containing $CdCl_2{\cdot}2.5H_2O$ (0 ~ 9 mM) and 10 mM of EGTA (ethylenebis(oxyethylenenitrilo)tetraacetic acid) were prepared, the log$K$ and pKa values of EGTA were taken from literatures[3] (log$K_{(Cd\text{-}EGTA)}$ = 16.5 (25°C, I = 0.1) and pKa_1 = 9.40, pKa_2 = 8.79, pKa_3 = 2.70). Protonation constants must be corrected upward by 0.11 when working at 0.1 M ionic strength, and the concentration of free $[Cd^{2+}]$ was calculated using reported method.[4]

$$[Cd^{2+}]_{free} = [Cd^{2+}]_{total}/K'_{(CdL)}[L]_{free}$$

$$K'_{(CdL)} = K_{(CdL)}/\alpha_M\alpha_L$$

$$\alpha_M = 1+10^{(pH\text{-}pKa1)},\ \alpha_L = 1+10^{(pKa1-\ pH)}+10^{(pKa1+pKa2-2pH)}+10^{(pKa1+pKa2+pKa3-3pH)}$$

$$[L]_{free} \approx [L]_{total} - [Cd^{2+}]_{total}$$

The fluorescence ratio F_{587}/F_{493} of each solution was measured, and fitted to the following equation (eq. 1[5]) which gave a K_d of 25 pM.

$$R = (R_{min}\, K_d + R_{max}\, [Cd^{2+}]_{free})/(K_d + [Cd^{2+}]_{free}) \qquad \text{(Eq. 1)}$$

In the above equation, R is the fluorescence ratio of F_{587}/F_{493}, R_{max} is the maximum fluorescence ratio of F_{587}/F_{493}, R_{min} is the fluorescence ratio of F_{587}/F_{493} with no addition of Cd^{2+}, and $[Cd^{2+}]_{free}$ is the free Cd^{2+} concentration.

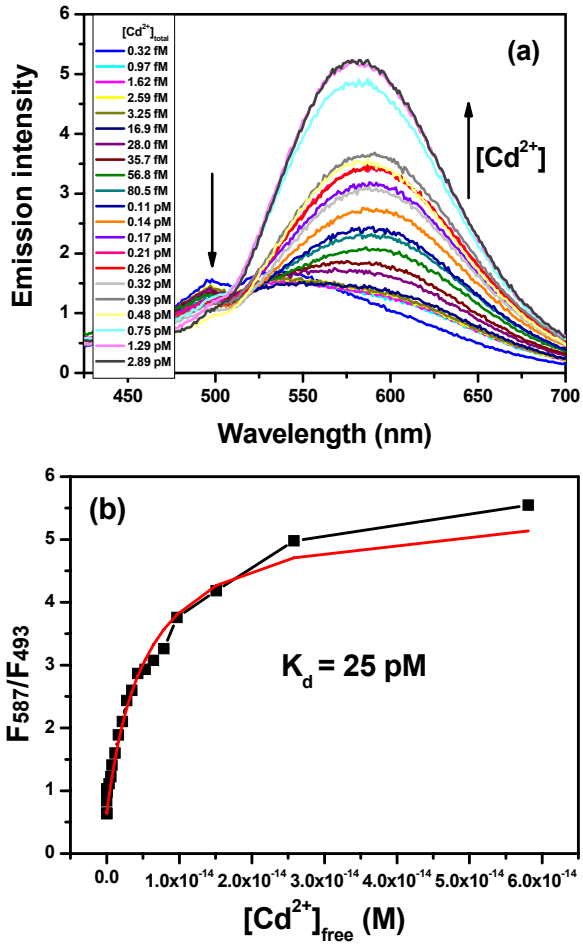


Figure S6. (a) Emission spectra of **DBITA** with the excitation at 362 nm in Cd^{2+}/EGTA buffered system (50 mM HEPES, pH 7.2, 0.1 M KNO_3, 10 mM EGTA); (b)Fluorescence ratio of **DBITA** (1×10^{-5} M) as a function of the concentration of free Cd^{2+} in 50 mM HEPES buffer (pH 7.2, 0.1 M KNO_3) with 10 mM EGTA and 0 - 9 mM Cd^{2+}. All data were expressed as the fluorescence ratio (587nm/493 nm).

S5. Determination of quantum yields

Fluorescence quantum yield of **DBITA** and Cd^{2+}/**DBITA** complex were determined in aqueous solutions (0.1 M KNO_3, 50 mM HEPES, pH = 7.2) by using quinine sulfate solution (Φ_f= 0.546, 0.5 M H_2SO_4) as references. The quantum yields were calculated using Eq.2:

$$\Phi_u = [(A_s F_u n^2)/ (A_u F_s n_0^2)]\Phi_s. \qquad \text{(Eq.2)}$$

Where A_s and A_u are the absorbance of the reference and sample solution at the reference excitation wavelength, F_s and F_u are the corresponding integrated fluorescence, n and n_0 are the refractive indexes of the solvents for the sample and reference solutions. Absorbance of samples and references at their respective excitation wavelengths was controlled to be lower than 0.05.

S6. Cell culture methods and confocal imaging

The confocal imaging was carried out with laser confocal microscope equipped with a 60 × oil-immersion objective using a dual-channel mode when excited at 405 nm, and the band paths are 460-510 and 560-610 nm, respectively. The imaging of HeLa cells was finished by Olympus FV10-ASW, while the imaging of macrophage cells by Zeiss LSM710.

For HeLa cells: HeLa cells were cultured in Dulbecco's Modified Eagle Medium supplemented with 10 % fetal bovine serum, penicillin (100 units/ml), streptomycin (100 mg/ml) and 5% CO_2 at 37 °C. After removing the incubation media and rinse with 1× PBS for three times, the cells were stained by **DBITA** by incubating the cells in 10 μM **DBITA** solution for 20 min at room temperature. Then, the cells was washed three times with PBS and imaged with confocal microscope. For the imaging of HeLa cells with exogenous Cd^{2+}, the exogenous Cd^{2+} was introduced by incubating the cells with 50 μM $CdCl_2$ solution for 2 h (Prepared by diluting 5 mM $CdCl_2$ stock solution with 1× PBS). After the imaging, the cells of exogenous Cd^{2+} were further treated with 100 μM TPEN solution (prepared by diluting the TPEN stock solution with 1× PBS) to scavenge the intracellular Cd^{2+}. Then the cells were rinsed with 1× PBS and imaged.

For macrophage cells: Murine peritoneal macrophages were isolated from 6- to 12-week-old C57BL/6J mice by using a reported protocol.[6] After cultured on coverslips for 2 h, the floating cells were removed by extensive washing, and the attached cells were maintained in RPMI 1640 medium (Invitrogen) containing 10% fetal bovine serum, penicillin (100 units/ml), streptomycin (100 mg/ml) and 5% CO_2 at 37 °C. After removing the incubation media and rinse with 1× PBS for three times, the cells were stained by incubating the cells in 10 μM **DBITA** solution for 20 min at room temperature. Then, the cells was washed three times with PBS and imaged by confocal microscope. For the imaging of macrophage cells with exogenous Cd^{2+}, the exogenous Cd^{2+} was introduced by incubating the cells with 10 μM $CdCl_2$ solution for 1.5 h (Prepared by diluting 5 mM $CdCl_2$ stock solution with 1× PBS). After the imaging, the cells of exogenous Cd^{2+} were further treated with 100 μM TPEN solution (prepared by diluting the TPEN stock solution with 1× PBS) to scavenge the intracellular Cd^{2+}. Then the cells were rinsed with 1× PBS and imaged.

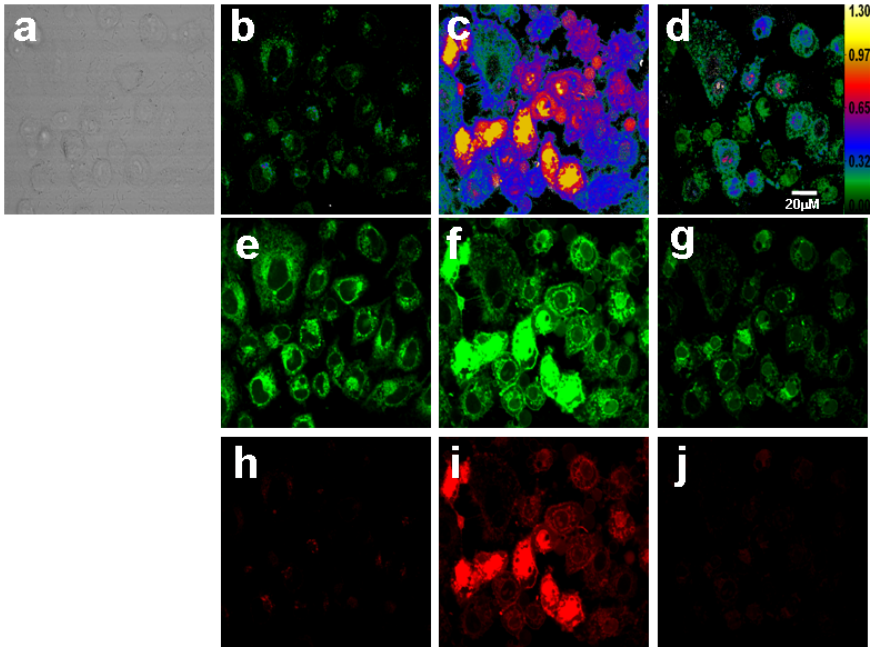


Figure S7. Confocal fluorescence images of intracellular Cd^{2+} in HeLa cells with **DBITA**-staining. HeLa cells incubated with **DBITA** (10 μM) at 25 °C for 20 min (b, e, h); the stained cells were exposed to 50 μM $CdCl_2$ solution at 25 °C for 2 h, followed by washing with **DBITA** solution (c, f, i), the cells in c, f and i further treated by TPEN solution (25 μM, 10 min, d, g, j). (a) Bright-field transmission images. (e, f, g) Fluorescence images obtained according to the emission collected at 460–510 nm. (h, i, j) Fluorescence images obtained according to the emission collected at 560–610 nm. (b, c, d) Ratiometric images generated from (h, i, j) and (e, f, g). λ_{ex}, 405 nm.

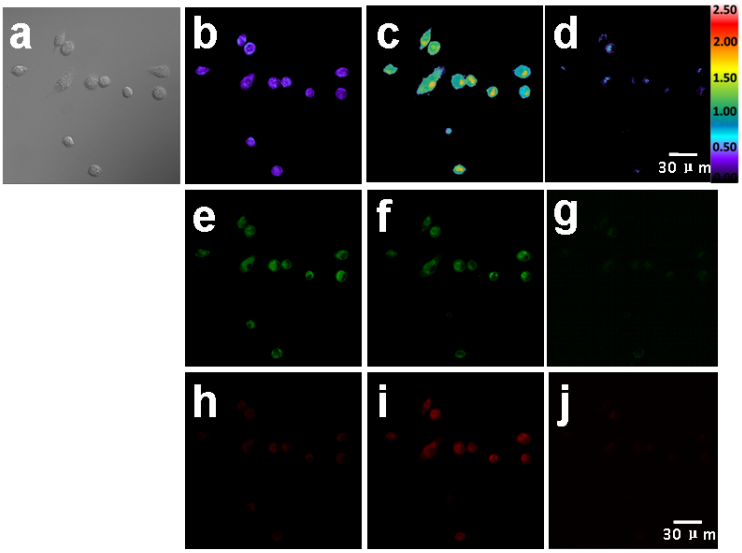


Figure S8. Confocal fluorescence images of intracellular Cd^{2+} in macrophage cells with **DBITA**-staining. Macrophage cells incubated with **DBITA** (10 μM) at 25 °C for 20 min (b, e, h); the stained cells were exposed to 10 μM $CdCl_2$ solution at 25 °C for 1.5 h, followed by washing with **DBITA** solution (c, f, i), the cells in c, f and i further treated by TPEN solution (25 μM, 10 min, d, g, j). (a) Bright-field transmission images. (e, f, g) Fluorescence images obtained according to the emission collected at 460–510 nm. (h, i, j) Fluorescence images obtained according to the emission collected at 560–610 nm. (b, c, d) Ratiometric images generated from (h, i, j) and (e, f, g). λ_{ex}, 405 nm.

S7. References

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