Electronic Supplementary Information

for

A highly sensitive and selective ratiometric Cd\(^{2+}\) fluorescent sensor for distinguishing Cd\(^{2+}\) from Zn\(^{2+}\) based on both fluorescence intensity and emission shift

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1. Materials and Methods.

Cationic compounds such as LiCl, NaCl, KCl, MgCl₂, CaCl₂, FeCl₃, ZnCl₂, CdCl₂, La(NO₃)₃, Ni(OAc)₂, Cu(OAc)₂, Mn(OAc)₂, Pb(OAc)₂, Hg(OAc)₂ and Ag(OAc) were purchased from Shanghai Chemical Co. and used without further purification. N-hexyl-3, 6-diodocarbazole\(^1\) and 5-bromo-8-tert-butyldimethylsilyloxy-2-methyl-quinoline\(^2\) were prepared according to the literature. Et₃N and 1,4-Dioxane were freshly distilled over appropriate drying agents. Reactions that required oxygen-free conditions were carried out under Argon atmosphere using standard Schlenk techniques. All the other reagents were purchased and used without further purification.

Fluorescence spectra measurements were performed on a Shimadzu RF-5301PC spectrofluorophotometer. Absorption spectra were determined on a Pgeneral UV-Vis TU-1901 Spetrophotometer. \(^1\)H and \(^1\)C NMR spectra were taken on a Bruker AVANCE II spectrometer with TMS as an internal standard and CDCl₃ or DMSO-d₆ as solvent. MS were performed on a ProteomeX-LTQ spectrometer. All spectra were recorded at room temperature (temperature controlled at 25 ± 3 °C).

2. Synthesis and characterization.

Synthesis of (4)

N-hexyl-3, 6-diodocarbazole (0.75 g, 1.5 mmol), pinacolborane (0.7 mL, 4.5 mmol), Et₃N (1.25 mL, 8.5 mmol) and PdCl₂(dppf) (0.05 g, 0.06 mmol) were dissolved in anhyd 1,4-dioxane (10 mL) under Ar atmosphere and the solution was stirred overnight at 90 °C. After cooling to room temperature, the mixture was poured into water (50 mL) and extracted with Et₂O (3 × 20 mL). The combined organic layers were dried (Na₂SO₄) and chromatographed on silica gel (petroleum ether: ethyl acetate = 20:1, v/v). Then the resulting light yellow solid was purified by recrystallization from hexane to give 4 (0.33 g) as white solid, yield: 44%. \(^1\)H NMR (400 MHz, CDCl₃): δ= 8.66(s, 2H), 7.90(d, 2H, J=8.0Hz), 7.39(d, 2H, J=8.0Hz), 4.30(t, 2H, J=7.2Hz), 1.85(m, 2H), 1.39(s, 24H), 1.35–1.27(m, 6H), 0.84(t, 3H, J=7.2Hz). \(^1\)C NMR (100 MHz, CDCl₃): δ= 142.6, 131.9, 128.0, 122.8, 108.1, 83.5,
43.1, 31.5, 28.8, 26.8, 24.9, 22.5, 13.9. MS: m/z = 504.22.

Synthesis of (2)

N-Hexyl-3,6-bis(4,4,5,5,-tetramethyl-1,3,2-dioxaborolan-2-yl)carbazole (0.30 g, 0.6 mmol), 5-bromo-8-tert-butyl(dimethyl)silyloxy-2-methyl-quinoline (0.46 g, 1.32 mmol), Pd(PPh₃)₄ (0.07 g, 0.06 mmol), and Na₂CO₃ (1.27 g, 12 mmol) were added to a flask under Ar atmosphere. A solvent mixture of toluene (12 mL), water (6 mL), and ethanol (3 mL) was degassed and added to the reaction mixture. The resulting mixture was refluxed with vigorous stirring for 24 h. After cooling to room temperature, the mixture was poured into water (50 mL) and extracted with Et₂O (3 × 20 mL). The combined organic layers were dried (Na₂SO₄) and chromatographed on silica gel (petroleum ether: ethyl acetate = 10:1, v/v). Then the resulting solid was purified by recrystallization from hexane to give 1 (0.32 g) as light yellow solid, yield: 70%. ¹H NMR (400 MHz, CDCl₃): δ = 8.18(d, 2H), 8.14(s, 2H), 7.55(m, 4H), 7.40(d, 2H), 7.22-7.16 (m, 4H), 4.41(t, 2H), 2.74(s, 6H), 2.03-1.95 (m, 2H), 1.54-1.25 (m, 8H), 1.09(s, 18H), 0.91(t, 3H), 0.33(s, 12). ¹³C NMR (100MHz, CDCl₃): δ=156.79, 151.32, 141.33, 140.12, 134.74, 133.64, 130.76, 128.22, 127.05, 126.42, 122.89, 121.94, 121.79, 117.60, 108.61, 43.48, 31.65, 29.14, 27.09, 26.02, 25.66, 24.73, 22.61, 18.99, 14.05. MS: m/z = 794.46.

Synthesis of (1)

To a 50 mL flask, containing 2 (0.2 g, 0.25 mmol) dissolved in tetrahydrofuran (20 mL) was added a THF solution of 1 M [NBu₄]F (0.8 mL). The mixture was stirred for 1 h at room temperature. After removal of the solvent in vacuum, the resulting solid was purified by recrystallization from methanol to give 1 (0.13 g) as white solid, yield: 92%. ¹H NMR (400 MHz, DMSO-d6): δ= 9.39(s, 2H), 8.28(d, 2H, , J=1.6Hz), 8.20(d, 2H, J=8.8Hz), 7.77(d, 2H, J=8.4Hz), 7.55-7.52(dd, 2H, J=1.6, 1.6Hz), 7.42(s, 2H), 7.41(d, 2H, J=1.2Hz), 7.16(d, 2H, J=8.0Hz), 4.51(t, 2H, J=6.8Hz), 2.70(s, 6H), 1.90-1.86(m, 2H), 1.42-1.28(m, 6H), 0.88-0.84(t, 3H, J=6.8Hz). ¹³C NMR (100MHz, DMSO-d6): δ= 156.71, 150.89, 140.27, 137.82, 135.31, 131.58, 130.65, 128.32, 127.65, 125.47, 123.08, 122.69, 121.95, 109.58, 108.85, 43.61, 31.78, 29.26, 27.22, 24.88, 22.74, 14.18. MS: m/z = 566.30.
3. Fluorometric Analysis.

The quantum yields of sensor 1 in different solvents were determined according to the following equation:

$$\Phi_u = \Phi_s \frac{F_u A_u n_u^2}{F_s A_s n_s^2}$$

Where $\Phi$ is quantum yield; $F$ is integrated area under the corrected emission spectra; $A$ is absorbance at the excitation wavelength; $n$ is the refractive index of the solution; the subscripts $u$ and $s$ refer to the unknown and the standard, respectively. Quinine bisulfate in 0.05 M H$_2$SO$_4$ solution was used as the standard, which has a quantum yield of 0.55.

**Fig. S1.** Ratiometric calibration curves $F_{525\text{nm}}/F_{443\text{nm}}$ as a function of Cd$^{2+}$ concentrations in HEPES (0.01 M) solution (dioxane/water = 4:1, v/v, pH 7.4). [1] = 20 μM. $\lambda_{ex} = 360$ nm.
Fig. S2. Fluorescence emission changes of sensor 1 at 525 nm vs Cd$^{2+}$ concentration from 0 to 0.2 μM in HEPES (0.01 M) solution (dioxane/water = 4:1, v/v, pH 7.4). [1] = 20 μM. $\lambda_{ex} = 360$ nm.

Fig. S3. Fluorescence spectra of sensor 1 + ZnCl$_2$ in HEPES (0.01 M) solution (dioxane/water = 4:1, v/v, pH 7.4) in the presence of increasing Zn$^{2+}$ concentrations. [1] = 20 μM. $\lambda_{ex} = 360$ nm.
**Fig. S4.** Fluorescence emission changes of sensor 1 at 555 nm vs Zn\(^{2+}\) concentration in HEPES (0.01 M) solution (dioxane/water = 4:1, v/v, pH 7.4). [1] = 20 μM. \(\lambda_{ex} = 360\) nm.

**Fig. S5.** Fluorescence spectra of 1 in the presence of various metal ions (100 equiv of Na\(^+\), K\(^+\), 10 equiv Mg\(^{2+}\), Ca\(^{2+}\), and 1.0 equiv Li\(^+\), Ag\(^+\), Zn\(^{2+}\), Cd\(^{2+}\), Hg\(^{2+}\), Ni\(^{2+}\), Cu\(^{2+}\), La\(^{3+}\), Mn\(^{2+}\), Pb\(^{2+}\), Fe\(^{3+}\)) in HEPES (0.01 M) solution (dioxane/water = 4:1, v/v, pH 7.4). [1] = 20 μM. \(\lambda_{ex} = 360\) nm.
**Fig. S6.** Fluorescence intensity of sensor 1, sensor 1 + 1.0 equiv Cd$^{2+}$ and sensor 1 + 1.0 equiv Zn$^{2+}$ at 525 nm recorded at various pH values in HEPES (0.01 M) solution (dioxane/water = 4:1, v/v, pH 7.4). [1] = 20 μM. $\lambda_{ex}$ = 360 nm.

**Fig. S7.** Fluorescence spectra of sensor 1 in dioxane/water mixtures with different water contents. [1] = 20 μM. $\lambda_{ex}$ = 360 nm.
**Fig. S8.** Fluorescence intensity of sensor 1, 1-Cd$^{2+}$ and 1-Zn$^{2+}$ at 525 nm recorded in dioxane/water mixtures with different water contents. [1] = 20 μM. $\lambda_{ex} = 360$ nm.

4. Job’ plots.

**Fig. S9.** Job’ plots of changes in fluorescence intensity at varying mole ratios of 1 and (a) Cd$^{2+}$, (b) Zn$^{2+}$ in HEPES (0.01 M) solution (dioxane/water = 4:1, v/v, pH 7.4). [M$^{2+}$]+[1] = 20 μM, $\lambda_{ex}$=360 nm.
5. Photographs.

Fig. S10. Naked eye colour changes and under UV lamp fluorescent color changes observed for 1 upon addition of one mole equivalent of Cd$^{2+}$ and Zn$^{2+}$ in HEPES (0.01 M) solution (dioxane/water = 4:1, v/v, pH 7.4). [1] = 20 μM. $\lambda_{ex}$ = 360 nm.

6. UV-visible Absorbance.

Fig. S11. Absorbance changes of sensor 1 at 345 nm vs Cd$^{2+}$ concentration in HEPES (0.01 M) solution (dioxane/water = 4:1, v/v, pH 7.4). [1] = 20 μM.
**Fig. S12.** Absorbance spectra of sensor 1 + ZnCl₂ in HEPES (0.01 M) solution (dioxane/water = 4:1, v/v, pH 7.4) in the presence of increasing Zn²⁺ concentrations. \([1] = 20 \text{ μM}\).

**Fig. S13.** Absorbance changes of sensor 1 at 345 nm and 400 nm vs Zn²⁺ concentration in HEPES (0.01 M) solution (dioxane/water = 4:1, v/v, pH 7.4). \([1] = 20 \text{ μM}\).
7. NMR Data.

$^1$H NMR (CDCl$_3$, 400 MHz) spectrum of 4

$^{13}$C NMR (CDCl$_3$, 400 MHz) spectrum of 4
$^1$H NMR (CDCl$_3$, 400 MHz) spectrum of 2

$^{13}$C NMR (CDCl$_3$, 400 MHz) spectrum of 2
\(^1\)H NMR (DMSO-d\(_6\), 400 MHz) spectrum of 1

\[^{13}\]C NMR (DMSO-d\(_6\), 400 MHz) spectrum of 1

References: