Supplementary Information

for

A turn-on fluorescent Fe^{3+} sensor derived from anthracene-bearing bisdien macrocycle and its intracellular imaging application

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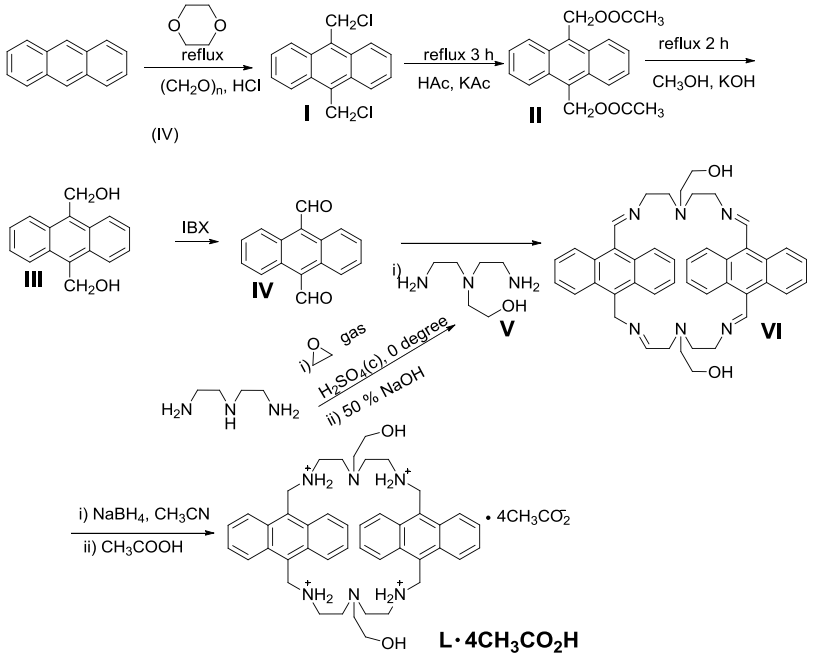
S1. Materials, general methods and instrumentations

Solvents and common reagents were commercial available and were used without further purification. All solvents for spectroscopic study were of spectrum grade, and the deionized water from MilliQ system (> 18 MΩ) was used. All fluorescence spectra were recorded using Horiba Scientifc Fluoromax-4 fluorescence spectrophotometer, while the UV/Vis absorption spectra were recorded on a LAMBDA-35 spectrophotometer. All NMR spectra were recorded using Bruker DRX-500 or Bruker DRX-300. pH values were record using sartorius PB-10 meter. Melting points were measured with X-4 melting point apparatus, and uncorrected. The electrospray ionization mass (ESI-MS) data was determined using a LCQ Fleet electro-spray mass spectrometer (Thermo Finnigan).

S2. Synthesis and characterization of sensor L.



Scheme S1: Synthesis of **L**

Synthesis of 9,10-bis(chloromethyl)anthracene (I)

After mixing 1, 4-dioxane (180 mL) and concentrated hydrochloride (30 mL) with stirring, the mixed solvent was saturated with hydrochloride via passing hydrochloride gas into the mixture with stirring for 20 minutes. Then anthracene (22.89 g, 128.4 mmol) and paraformaldehyde (19.43 g) were added into this mixture and heated to 145 °C with stirring, and HCl gas was passed continually into the mixture for 2 h. The mixture was refluxed with stirring for an additional 3 h. After filtration and washing with dioxane, the yellow powder crud was obtained. The purified compound **I** (19.74 g) was obtained as yellow crystal by recrystallization from toluene. Yield: 51%. M.p. 250-251°C.

Synthesis of anthracene-9, 10-diylbis (methyl) diacetate (II)

Compound **I** (0.50 g, 1.8 mmol) and KAc (1.428 g, 14.55 mmol) were mixed in acetic acid (40 mL) and refluxed at 120 °C for 3 h. Then the mixture was poured into the cold water (150 mL) and yellow precipitate was formed,[1] and compound **II** (0.55 g) was obtained as solid via filtration and drying in vacuo. Yield. 93%. M. p. 218-222 °C.

Synthesis of anthracene-9,10-diyldimethanol (III)[2]

Compound **II** (0.55 g, 1.55 mmol) and KOH (1.65 g, 29.5 mmol) were dissolved in methanol (42 mL) and refluxed for 2 h. Then the solution was cooled to room temperature. The solid product (**III**, 0.33g) was obtained via filtration and washing with water. Yield, 91%, M. p. 280-281°C.[2]

Synthesis of anthracene-9,10-dicarbaldehyde (IV) [3]

Compound **III** (0.5 g, 2.1 mmol) and 3 equiv 2-iodoxybenzoic acid (IBX) were dissolved in 1, 2-dichloroethane and the mixture was heated to 80 °C for 3 h. Then the solution was cooled to room temperature, and the solvent was removed in vacuo. The residue was washed with water and compound **IV** was obtained as the orange crystals. Yield. 70%. M.p.146-148 °C. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 7.715 (q, 4H), 8.758 (q, 4H), 11.501 (s, 2H).

Synthesis of 1-bis(1-aminoethyl) amino ethanol (V) [4]

Diethylenetriamine (150 g, 1454 mmol) and concentrated sulfuric acid (93 ml) were mixed in water followed by the slowly addition of ethylene oxide (30 g, 681.0 mmol) in 1 h. Then, the mixture was stirred overnight. The mixture was poured into 400 g NaOH solution (50%). The precipitate was filtrated off and extracted with isopropyl alcohol. The solvent was removed in vacuo from the combined extract. The residue was distilled at reduced pressure and compound **V** was collected at 148 °C/11 mmHg. Yield, 13%. ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.576 (m, 4H), 1.649 (d, 1H), 1.745 (m, 4H), 3.559 (m, 1H).

Synthesis of L [5]

Compound **IV** (0.5 g, 2.1 mmol) was dissolved in acetonitrile (300 mL) and added dropwise in 1 h to an acetonitrile solution (60 mL) containing 0.5 mL of compound **V**. The reaction mixture was then stirred at room temperature for 1 day. The solid intermediate was separated from the mixture via filtration. Then this solid was suspended in methanol and heated to 45 °C. After that, sodium borohydride (1.0 g, 26.4 mmol) was added to the mixture in portion within 1 h. The mixture was stirred at room temperature overnight. After removing the solvent in vacuo, the residue was extracted by 10 mL $CHCl_3$ containing HAc (0.17 M). Crude product was obtained from water phase by evaporation, and recrystallization from ethanol afforded pure yellow product (**L**·$4CH_3CO_2H$), 0.6 g. Yield. 46%, ESMS (positive mode, m/z): calcd. 699.44, found 699.42 for $[\mathbf{L}+H]^{+}$; calcd. 355.23, found 355.50 for $[\mathbf{L}+2H]^{2+}$. ^{1}H NMR (500 MHz, CD_3OD, δ, ppm): 8.15 (d, J=10.0 Hz, 8H), 7.24 (d, J=10.0 Hz, 8H), 4.84 (s, 8H, Ar-CH_2*-NH-), 3.75 (t, J = 10.0 Hz, 4H, -NH-CH_2-CH_2*-OH), 3.44 (t, J =10.0 Hz, 8H, -NH-CH_2*-CH_2-), 3.06 (s, 8H, -NH-CH_2-CH_2*-), 2.81(t, J =10.0 Hz, 4H, -NH-CH_2*-CH_2-OH), 1.93(s, 12H, CH_3*COO^{-}). ^{1}H NMR signals at 3.33, 3.37 and 4.91 ppm are the solvent residual peaks. ^{13}C-NMR ($CHCl_3$ δ, ppm): 176.53, 129.87, 127.71, 125.86, 124.01, 59.68, 56.05, 51.02, 46.19, 43.48, 21.26. M.p. 178 - 180 °C.

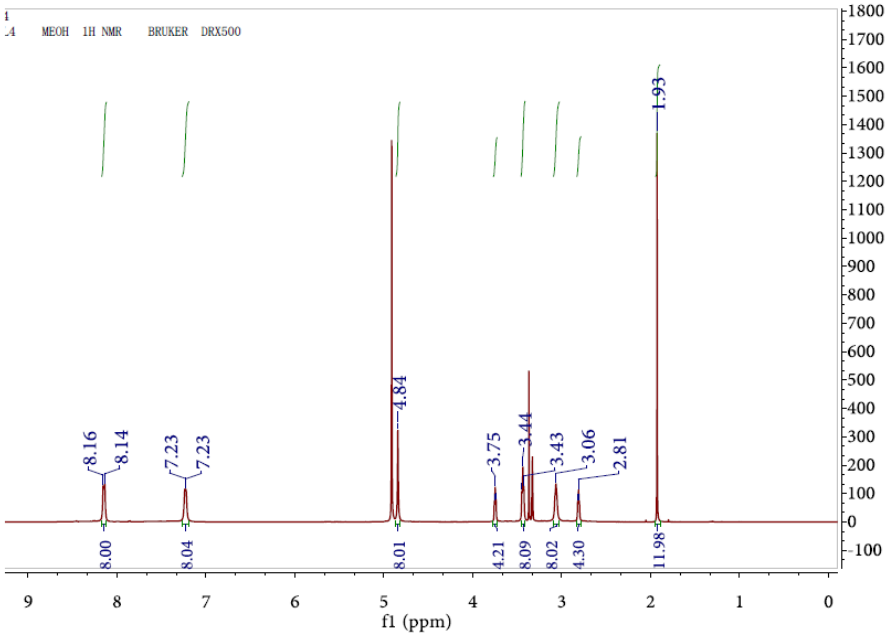


Fig. S1 ^{1}H-NMR spectrum of **L**·4CH_3CO_2H in CD_3OD.

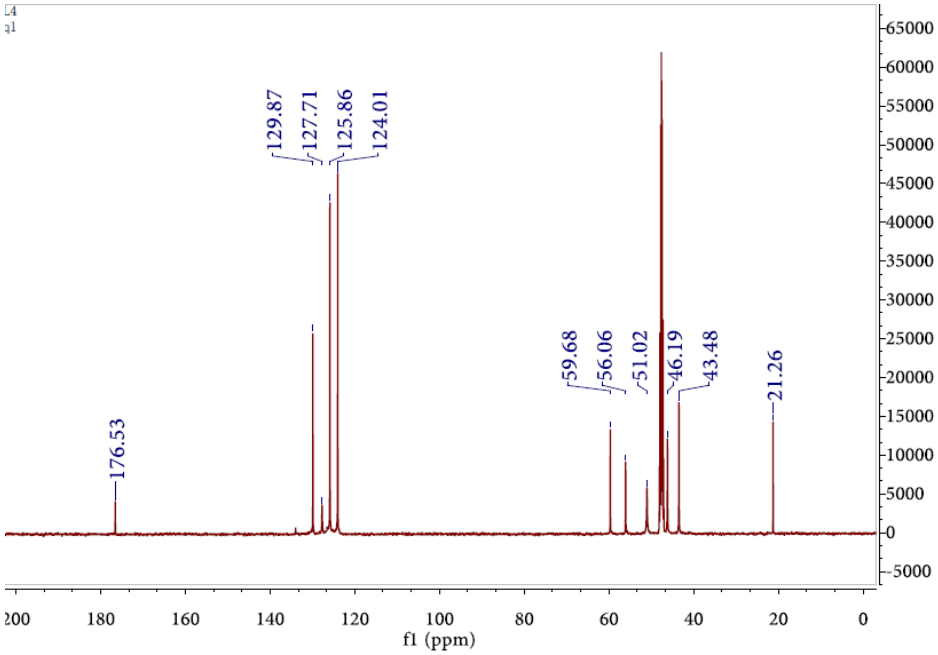


Fig. S2 ^{13}C-NMR spectrum of **L**·4CH_3CO_2H in CD_3OD

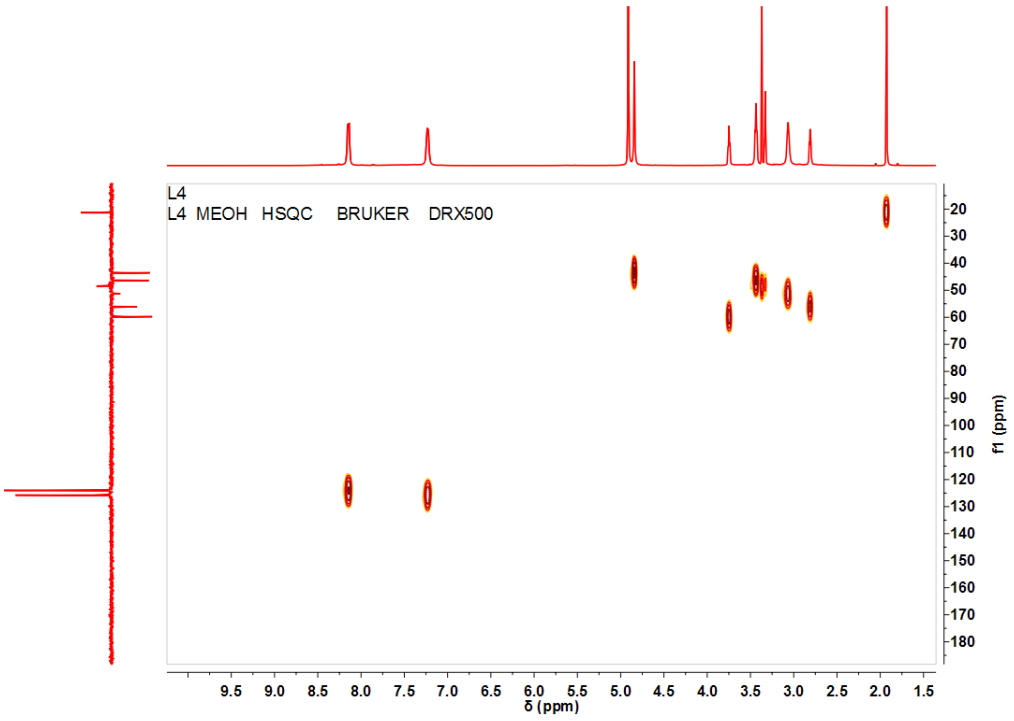


Fig. S3 $^{13}C/^{1}H$ HSQC spectrum of **L**·4CH_3CO_2H in CD_3OD

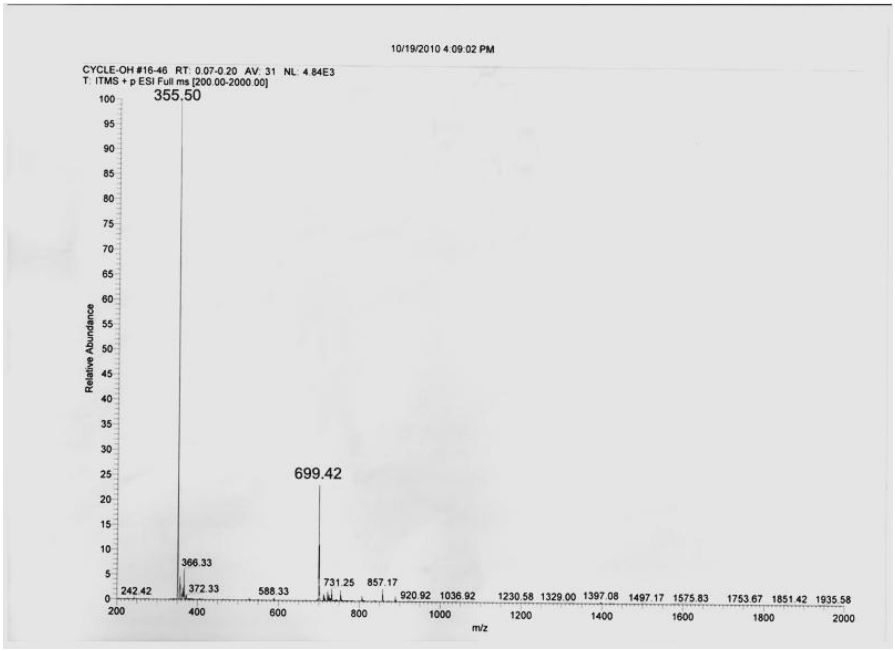


Fig. S4 ESI-MS spectrum of **L**·4CH_3CO_2H.

S3. Single crystal structure of L·4CH_3CO_2H·4H_2O·2$CDCl_3$

The single crystals suitable for X-ray structure resolution were obtained from the sensor solution (CD_3Cl) in NMR tube via very slow evaporation. The X-ray diffraction data were collected on a Smart APEX CCD diffractometer. The structure was resolved with SHELXL-97 programs.

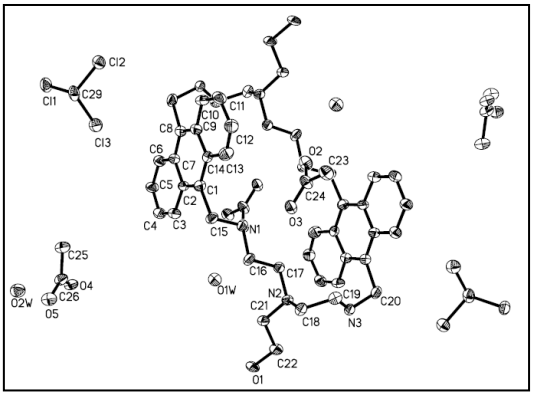


Fig. S5 ORTEP diagram of **L**·4CH_3CO_2H·4H_2O·2$CDCl_3$ (H-atoms were omitted for clarity).

Table S1. Crystal parameters and structure refinements for **L**·4CH_3CO_2H·4H_2O·2$CDCl_3$

Empirical Formula	C_{54} H_{80} C_{l6} N_6 O_{14}
Formula weight	1249.94
Temperature (K)	291(2)
Crystal size (mm^3)	0.20×0.22×0.28
Crystal system	Triclinic
Space group	P-1
a(Å)	10.5570(8)
b(Å)	12.6918(9)
c(Å)	12.8872(9)
α (°)	111.7430(10)
β (°)	96.6930(10)
γ(°)	95.4490(10)
V (Å^3)	1575.0(2)
Z	1
D_{calcd} *(g/ cm^3)*	1.318
F(000)	660
θ range (°)	1.75-26.00
Tot., ref. number	6044，8656
Limiting indices	-12⩽h⩽13, -15⩽k⩽13, -13⩽l⩽15
Observed data [I > 2σ(I)]	3043
$R_{(int)}$	0.0532
R_1,wR_2 [I > 2σ(I)]	0.0603, 0.1306
GoF on F^2	1.050
S	1.050

Table S2. Selected bond distances (Å) and angles (º) for **L**·4CH_3CO_2H·4H_2O·2$CDCl_3$

Bond lengths (Å)		Bond angles (º)	
C1 C15	1.607(5)	C9 C14 C1	119.3(4)
C15 N1	1.494(5)	C9C14C13	116.4(4)
C16 N1	1.497(5)	C14 C1 C15	119.4(4)
C16 C17	1.532(5)	C15 N1 C16	108.2(3)
C17 N2	1.471(5)	N1 C16 C17	110.4(3)
C18 N2	1.457(5)	C18 N2 C17	114.4(3)
C18 C19	1.524(5)	N2 C18 C19	109.2(3)
C19 N3	1.485(5)	N3C19C18	107.3(3)
C20 N3	1.500(5)	N3 C20 C8	108.9(3)
C20 C8	1.582(5)	C7C8C20	123.4(4)
C21 N2.	1.473(5)	C8 C7 C6	122.6(4)
C22 O1	1.403(4)	C8 C7 C2	120.1(4)
C23 C24	1.505(6)	C17 N2 C21	112.8(3)
C24 O3	1.236(5)	N2 C21 C22	112.6(3)
C24 O2	1.256(5)	C14 C9 C8	119.3(4)
		O1 C22 C21	109.5(3)
		C18N2C21	114.4(3)
		C9 C8 C20	115.8(4)

S4. UV-vis and fluorescence spectroscopic study of L

Stock solutions of Cd^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, K^{+}, Mg^{2+}, Ca^{2+}, Na^{+}, Zn^{2+}, Fe^{2+}, Al^{3+}, Cr^{3+}, Hg^{2+}, and Fe^{3+} were prepared via dissolving the related salts in the deionized water. The concentration of Cd^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, Fe^{2+}, Al^{3+}, Cr^{3+}, Hg^{2+}, Fe^{3+} is 1.2 mM and that for K^{+}, Mg^{2+}, Ca^{2+}, Na^{+} is 1 M. The sensor solution (8.7 μM) for spectroscopic study was prepared by dissolving **L** in Tris-HCl buffer (20 mM, pH7.20) containing 50% methanol (v/v). For all fluorescence spectra determination, the excitation wavelength was 373 nm. Both excitation and emission slit widths were 5 nm.

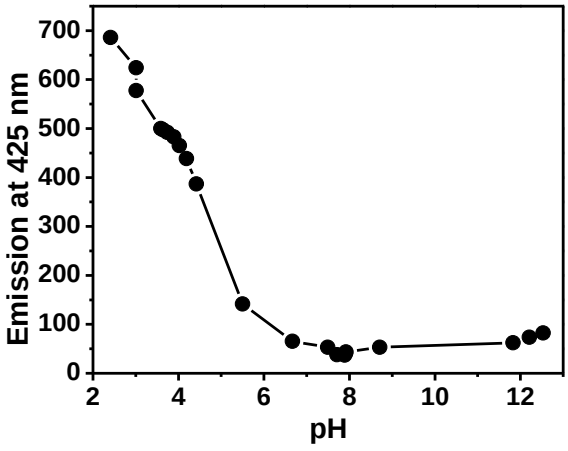


Fig. S6 Fluorescent pH titration profile of **L** (8.7 μM) in water containing 50% methanol according to the emission intensity at 425 nm (λ_{ex}=373 nm).

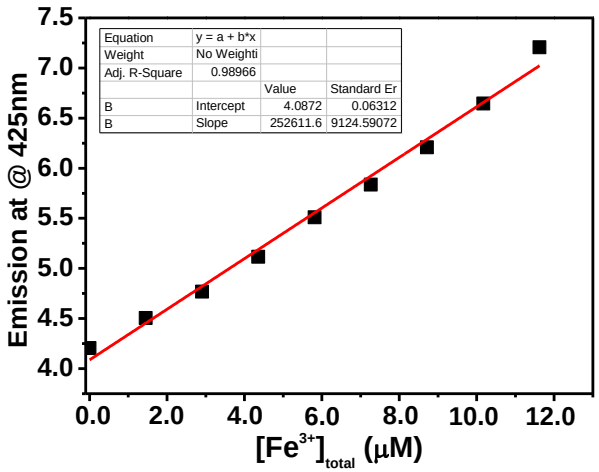


Fig. S7 Plot of emission intensity of **L** (8.7 μM) in Tris-HCl buffer (20 mM, pH 7.20) containing 50% CH_3OH (v/v) at 425 nm as a function of $[Fe^{3+}]_{total}$ in the range of 1.0×10^{-6} M to 1.0×10^{-5} M.

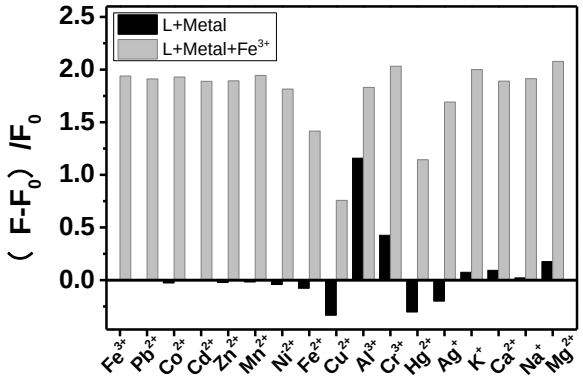


Fig. S8 Fluorescent response of **L** (8.7 μM) to Fe^{3+}, Pb^{2+}, Co^{2+}, Cd^{2+}, Zn^{2+}, Mn^{2+}, Ni^{2+}, Fe^{2+}, Cu^{2+}, Fe^{3+}, Al^{3+}, Cr^{3+}, Hg^{2+} (36 μM), and K^+, Na^+, Mg^{2+}, Ca^{2+} (1000 μM) in Tris-HCl buffer (20 mM, pH 7.20) containing 40% (v/v) methanol. The response in the presence of Fe^{3+} (36 μM) was shown in grey. $(F-F_0)/F_0$ was calculated according to the emission at 425 nm.

S5. Characterization of Fe^{3+}/L binding

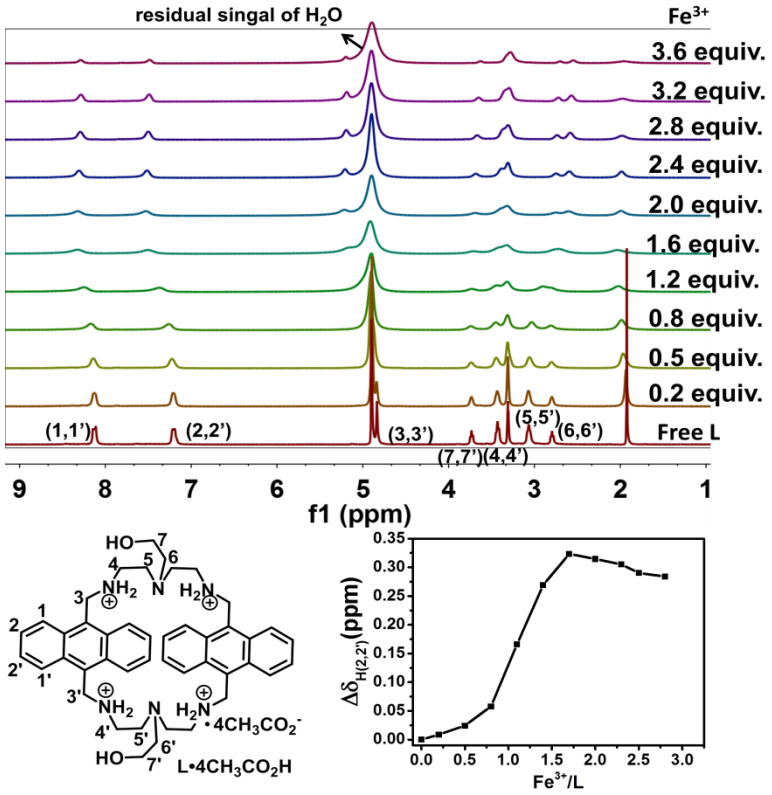


Fig. S9 (Upper) ^{1}H NMR spectra of **L** (0.01 M in CD_3OD) upon titration by a Fe^{3+} solution in CD_3OD (1 M). (Lower right) Titration profile according the change of the signal for $H^{2,2'}$.

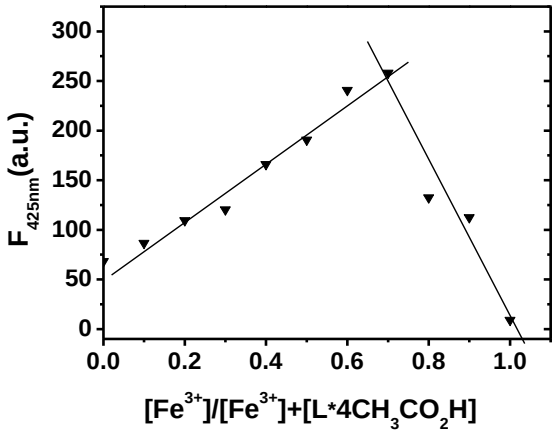


Fig. S10 Job's plot of Fe^{3+}/**L** binding in Tris-HCl buffer (20 mM, pH 7.20) containing 50% methanol (v/v) according to the emission at 425 nm. The total molar concentration of **L** and Fe^{3+} is 50 μM.

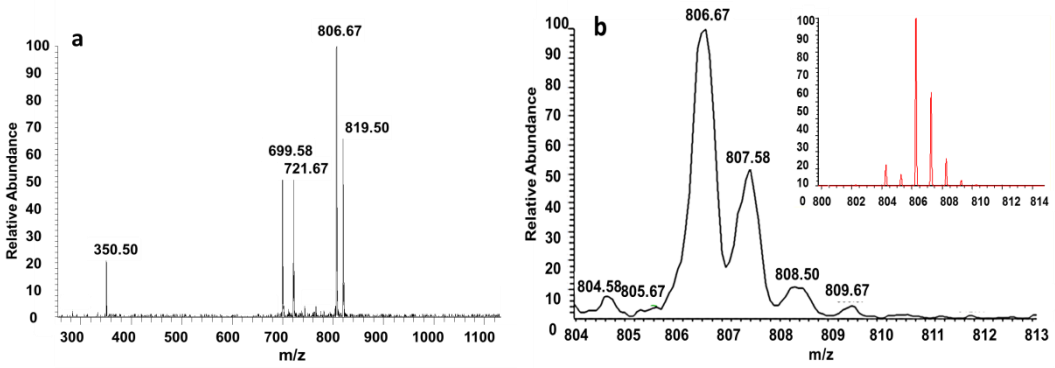


Fig. S11 (a) ESI—MS spectrum of **L** (8.7 μM) solution mixed with 4 equiv Fe^{3+}. (b) The related isotopic distribution pattern of signal m/z of 806.67. The red one is the simulated isotopic distribution pattern of $[\mathbf{L}+2Fe-4H]^{+}$. Signals at 350.50, 699.58, 721.67 and 819.50 can be assigned as $[\mathbf{L}+2H]^{2+}$ (calcd:350.23), $[\mathbf{L}+H]^{+}$ (calcd:699.43), $[\mathbf{L}+Na]^{+}$ (calcd:721.42) and $[\mathbf{L}+2CH_3COOH+H]^{+}$ (calcd:819.48) respectively.

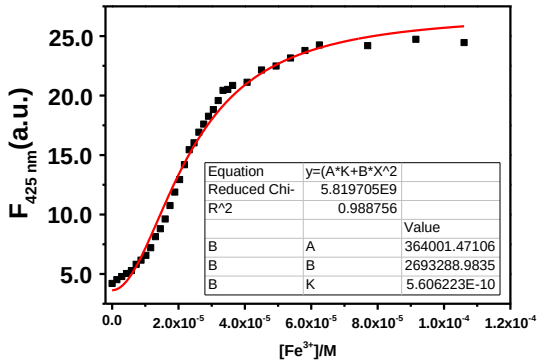


Fig. S12 Fluorescence intensity at 425 nm of **L** (8.7 μM) in Tris-HCl buffer (20 mM, pH 7.20) containing 50% (v/v) methanol as a function of Fe^{3+} concentration. The apparent dissociation constant (K_d) was obtained by fitting this titration profile with the following equation:[6]

$$F = \frac{F_{min}K_d + F_{max}[X]^n}{K_d + [X]^n} \qquad (1)$$

in which F is the fluorescence intensity of sensor at $[Fe^{3+}]$, F_{min}, the fluorescence intensity of free sensor at 425 nm, F_{max}, the fluorescence intensity of Fe^{3+}-bound sensor at 425 nm, and n, the number of Fe^{3+} bound per probe.

S6. Fluorescent response of L' to Fe^{3+}

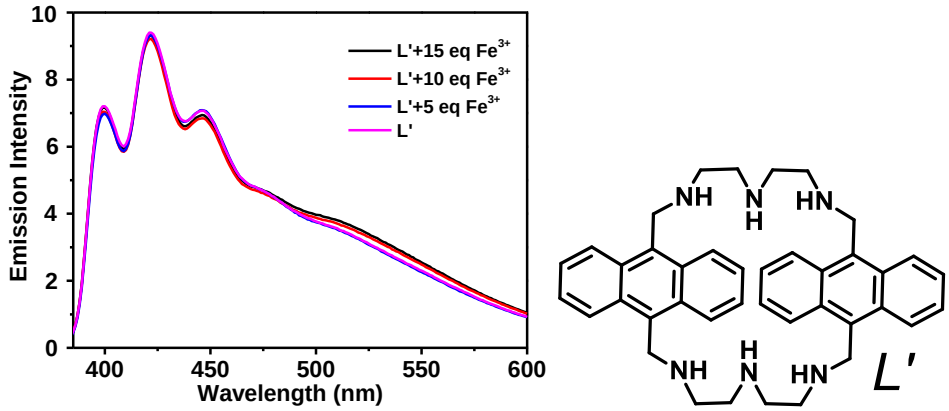


Fig. S13 Fluorescence spectra of **L'** (10.0 μM) in Tris-HCl buffer (20 mM, pH 7.20) containing 50% CH_3OH (v/v) in the presence of 0, 5, 10, and 15 equiv Fe^{3+}. λ_{ex}, 373 nm.

S7. Confocal fluorescence imaging of SKOV-3 and HeLa cells via L-Staining

SKOV-3 cells (1.0×10^5 cells mL^{-1}) were seeded on coverslip and incubated under 5% CO_2 at 37 °C. For the Fe^{3+} and Fe^{2+}-overloaded cells, the cells at 90% confluence were treated with 50 μM ferric citrate or ferrous ammonium sulfate dissolved in DMEM media. After 4 h of incubation, the medium was removed and the cells were washed with phosphate buffered saline (1× PBS) three times. Sensor solution for cell staining was prepared by diluting **L** stock solution (0.87 mM) with 1× PBS, and the final concentration was 8.7 μM. The cells were incubated with this diluted sensor solution at 37 °C for 30 min and imaged thereafter. The phase contrast image and fluorescence image were obtained respectively using confocal microscope (Zeiss LSM 710). After being washed with 1× PBS for 3 times, the cells were then incubated by 50 μM TPEN solution (prepared by diluting the 1 mM TPEN stock solution with 1× PBS) for 30 min at 37 °C. After the incubation, the cells were imaged again with the same imaging conditions. The normal SKOV-3 cells without the overloaded Fe^{3+} or Fe^{2+} were also imaged after **L**-staining with the same imaging conditions. All imaging experiments were excited at 405 nm, and the band path is 425-460 nm. The Fe^{3+}-overloaded and normal HeLa cells were also imaged in the same procedure.

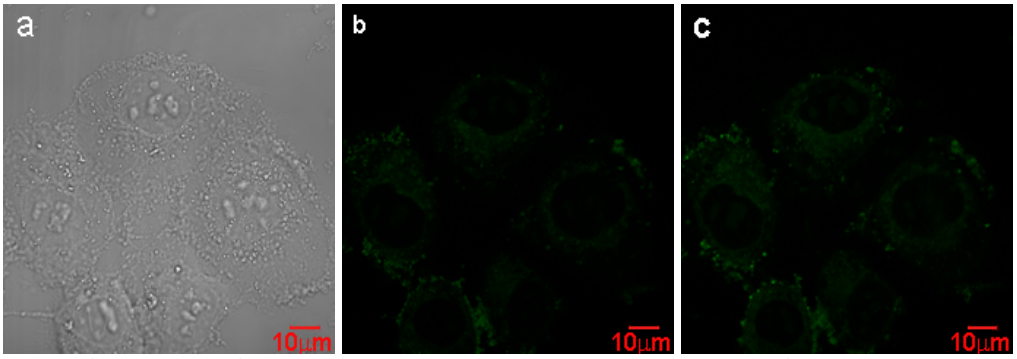


Fig. S14 Fluorescence image of SKOV-3 cells after incubation with **L** (8.7 μM) in 1× PBS at 37 °C. (**a**) Bright-field transmission image; (**b**) fluorescence image upon 20 min of incubation with **L**; (**c**) fluorescence image upon 30 min of incubation with **L**. λ_{ex}, 405 nm, band path 425-460 nm.

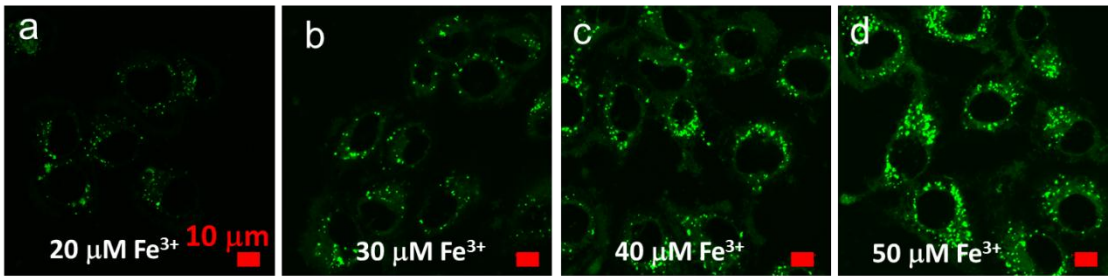


Fig. S15. Confocal fluorescence imaging of SKOV-3 cells incubated with 20 (**a**), 30 (**b**), 40 (**c**) and 50 μM Fe^{3+} (**d**) for 4 h, followed by PBS washing (3 times) and **L** incubation (8.7 μM in PBS buffer, 30 min, 37°C) in sequential. λ_{ex}, 405 nm, band path 425-460 nm. Scale bar, 10 μm.

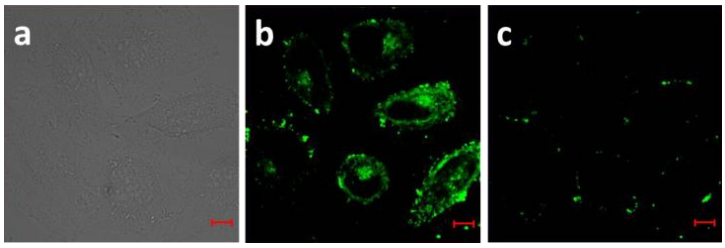


Fig. S16 Confocal fluorescence imaging of Fe^{3+}-overloaded HeLa cells stained by **L**. (**a**) Bright-field transmission image of cells incubated with Fe^{3+} (50 μM) for 4 h, followed by PBS washing (3 times) and **L** incubation (8.7 μM in PBS buffer, 30 min, 37°C) in sequential; (**b**) fluorescence image of cells in (**a**); (**c**) fluorescence image of cells in (**b**) followed by further incubation with TPEN (50 μM, 30 min, 37°C). λ_{ex}, 405 nm, band path 425-460 nm. Scale bar, 10 μm.

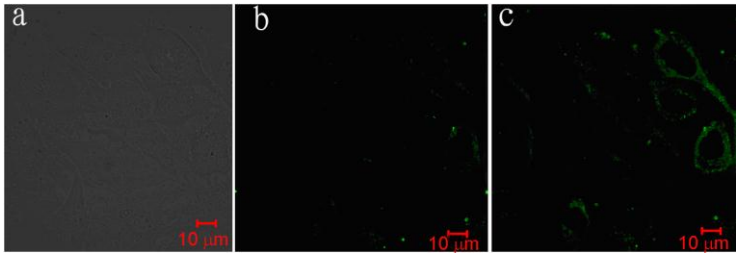


Fig. S17 Fluorescence image of HeLa cells after incubation with **L** (8.7 μM) in 1× PBS at 37 °C. (**a**) bright-field transmission image; (**b**) fluorescence image upon 20 min of incubation; (**c**) fluorescence image upon 30 min of incubation. λ_{ex}, 405 nm, band path 425-460 nm. Scale bar, 10 μm.

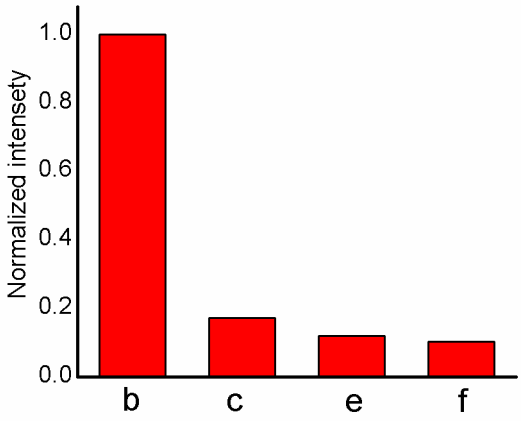


Fig. S18. Normalized fluorescence intensity of regions of interest shown in Fig.5.

S8. MTT Assay

HeLa cells and SKOV-3 cells (10^6 cells mL^{-1}) were respectively dispersed within replicate 96-well microtiter plates to a total volume of 100 μL $well^{-1}$. Plates were maintained at 37 °C in a 5% CO_2/95% air incubator for 24 h. Cells were then incubated for 24 h with different concentration of probe (0, 5, 10, 20, 40, and 80 μM) respectively. MTT (Sigma) solution (5.0 mg mL^{-1}, PBS) was then added to each well. After 4 h of incubation at room temperature, the MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. After that, the absorbance was measured at 570 nm in a TRITURUS microplate reader.

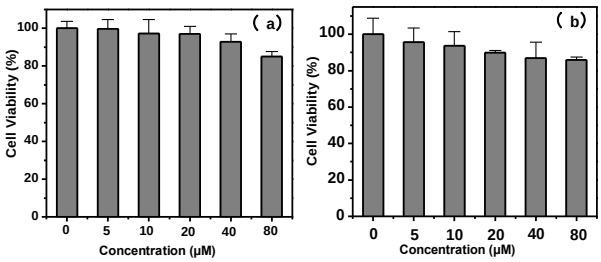


Fig. S19 Cytotoxicity of **L** against the HeLa (**a**) and SKOV-3 cells (**b**) determined by MTT assay after 24 h of incubation.

S9. References:

1 G.M. Badger, J.W. Cook, *J. Chem. Soc*., 1939, 802

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