Electronic Supporting Information

Rhodamine-based “off-on” fluorescent chemosensor for selective detection of Fe$^{3+}$ in aqueous media and its application in bioimaging

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Experimental

$^1$H and $^{13}$C NMR spectra were recorded on a Bruka Advance-III spectrometer (at 400 and 100 MHz, respectively) in CDCl$_3$. High-resolution mass spectra were recorded on a Bruka Autoflex mass spectrometer (MALDI-TOF). Fluorescent emission spectra and UV-vis spectra were collected on a PE LS50B and a Cary UV-100 spectrometer, respectively. All fine chemicals were used as received. CaSHII was synthesized using our published method [1].

Reference:

Synthesis of FIS1:

A solution of Rhodamine B (0.30 g, 0.62 mmol) in POCl$_3$ (5 mL) was refluxed for 5 h. The reaction mixture was then cooled and concentrated by evaporation. The obtained acid chloride which was dissolved in ClCH$_2$CH$_2$Cl (20 mL) together with CaSHII (0.14 g, 0.58 mmol) and triethylamine (0.7 mL). The mixture was stirred at room temperature for 12 h and was then concentrated by evaporation. The crude product was purified by silica gel column chromatography with EtOAc/$n$-hexane (1/3, v/v) to afford FIS1 as a pink solid (m.p. 225-227°C, 0.12 g, yield 33%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.91(1H, d, $J$ = 7.6 Hz ), 7.57-7.49 (2H, m), 7.20 (1H, d, $J$ = 7.6 Hz ), 6.57 (1H, d, $J$ = 8.8 Hz ),
6.42-6.25 (5H, m), 4.54-4.51 (1H, m), 3.40-3.24 (9 H, m), 3.15 (1H, d, J = 13.6 Hz),
1.79-1.75 (1H, m), 1.66-1.63 (2H, m), 1.46-1.44 (1H, m), 1.41-1.35 (1H, m), 1.15-1.11
(12H, m), 1.04-0.94 (5H, m), 0.82 (1H, s), 0.66-0.61(1H, m). $^{13}$C NMR (100 MHz,
CDCl$_3$) δ 164.3, 154.6, 150.3, 149.2, 148.9, 133.2, 130.9, 129.9, 129.1, 128.3, 124.5,
123.0, 108.3, 108.1, 107.0, 98.6, 98.0, 66.8, 66.4, 50.0, 48.0, 46.2, 44.6, 44.4, 36.0,
34.2, 26.2, 21.6, 19.7, 12.6, 12.4, 12.2 ppm.

HRMS (ESI): m/z calcd for C$_{48}$H$_{47}$N$_4$O$_4$S [M+H]$^+$: 655.3313; found, 655.3295.

$[\alpha]_D^{20} = -26.88$ (c=0.42, CHCl$_3$)
Fig. S1 $^1$H NMR spectrum of FIS1

DEPT-135

Fig. S2 $^{13}$C NMR (DEPT-135 mode) spectrum of FIS1

Fig. S3 $^{13}$C NMR spectrum of FIS1
Fig. S4 HRMS (ESI mode) of FIS1
**Fig. S5** Fluorescent spectra of **FIS1** (10 μM) upon addition of 3.5 equivalent of Fe$^{3+}$ in aqueous solution of acetonitrile of different composition (H$_2$O: ACN, v/v = 99:1; 90:10; 80:20; 70:30; 60:40; 50:50).

**Fig. S6** The pH effect of the probe (1 µM) in aqueous ACN solution (1:1, v/v) at different pH in response to 10 equivalent of Fe$^{3+}$.
Estimation of the LOD of the probe:

\[ 3 \times \text{standard deviation of the Fluorescent Ratio Value} \div \text{Slope of the Calibration Curve} \]

\[ = 3 \times 0.5546 \div 6.5153 \]

\[ = 0.2554 \mu M \]

**Fig. S7** The estimation of the LOD of **FIS1** by fluorescent titration with Fe\(^{3+}\) by the probe.
Fig. S8 Change of fluorescence spectra of the probe FIS1 (2 µM) in response to 10 equiv. of Fe$^{3+}$ over a period of 10 min (1:1 = H$_2$O:ACN, v/v).