A highly pH-sensitive Zn(II) chemosensor

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Synthesis of H$_2$L.

2,7-dichloro-4,5-bis(bis-(N-N’-phtalimide-ethylenamino)aminomethyl)-fluorescein (1)
Paraformaldehyde (1.20 g, 39.8 mmol) was added to a suspension of bis-phthalimide-diethylenetriamine (13.6 g, 37.3 mmol) in 170 ml of MeCN, and then stirred under reflux at 60°C for 30 min, before addition of a suspension of 2’,7’-dichlorofluorescein (5.0 g, 12.4 mmol) in 80 ml of MeCN:H$_2$O (1:1). After being stirred overnight at 60°C, the mixture was allowed to cool to room temperature. Solvent removal in vacuum afforded a red solid residue, which was suspended in CH$_2$Cl$_2$ and filtered. Purification by chromatography on silica (100:3 CH$_2$Cl$_2$:MeOH, Rf=0.5) gave a pale pink powder (3.50 g, 3.04 mmol, 25% yield).

$^1$H-NMR (CDCl$_3$, 300MHz): $\delta$ 7.89 (d, $J = 7.1$ Hz, 1H), 7.63-7.56 (m, 19H), 6.39 (s, 2H), 4.28 (s, 4H), 3.87 (m, 8H), 3.03 (m, 8H). $^{13}$C-NMR (CDCl$_3$, 75 MHz): $\delta$ 168.1, 155.2, 147.7, 135.2, 133.9, 133.6, 131.9, 130.0, 127.6, 126.6, 125.1, 124.2, 123.3, 123.0, 117.0, 110.4, 108.9, 51.3, 50.6, 34.4. Elemental analysis calcd. for C$_{62}$H$_{46}$Cl$_2$N$_6$O$_{13}$: C: 64.53, H: 4.02, N: 7.28; found: C: 64.4, H: 4.0, N: 7.4.

2,7-dichloro-4,5-bis(ethylenamino)aminomethyl)-fluorescein (H$_2$L)
Hydrazine monohydrate (50%, 40 ml) was added to a suspension of 1 (750 mg, 0.65 mmol) in 400 ml of EtOH and after few minutes the formation of a red precipitate is observed. The mixture was refluxed for 12 h in the dark, cooled to room temperature, filtered, washed with EtOH and dried under vacuum in presence of KOH, yielding 290 mg (0.44 mmol, 67% yield) of a deep red solid.

$^1$H-NMR (D$_2$O, pH 2, 300 MHz) : $\delta$ 7.66 (d, $J = 6.6$Hz, 1H), 7.25 (t, $J = 6.5$ Hz, 2H), 6.87 (d, $J = 6.6$ Hz, 1H), 6.74 (s, 2H), 4.43 (s, 4H), 3.39 (t, $J = 7.1$ Hz, 8H), 3.10 (t, $J = 7.1$ Hz, 8H). $^{13}$C-NMR (D$_2$O, pH 2, 75 MHz): $\delta$ 168.9, 163.2, 152.2, 138.6, 134.4, 130.9, 129.3, 128.2, 127.9, 123.1, 114.4, 104.8, 50.6, 49.7, 34.1. ESI-MS [H$_2$L+H]$^+$, 632. Elemental analysis calcd. for C$_{30}$H$_{36}$Cl$_2$N$_6$O$_5$·2H$_2$O: C: 53.97, H: 6.04, N: 12.59; found: C: 53.49, H: 5.89, N: 12.79.
**Figure S1.** Absorption spectra of 2’,7’-dichlorofluorescein in aqueous solution at different pH values ([2’,7’-dichlorofluorescein] = 1.67 x 10^{-5} M)

**Figure S2.** Fluorescence emission spectra of 2’,7’-dichlorofluorescein in aqueous solution at different pH values ([2’,7’-dichlorofluorescein] = 3.25 x 10^{-7} M, λ_{exc} 472 nm).
Figure S3. Minimized conformations of [CdL(H$_2$O)] complex. Bond distances (Å): Cd-N1, 2.408; Cd-N2, 2.415; Cd-N3, 2.392; Cd-O1, 2.158; Cd-Ow1, 2.377.
Figure S4. pH dependence of the absorbance at 505 nm (Cd(II), Pb(II)) or 498 nm (Cu(II)) as a function of pH compared to the distribution curves of the complexes (solid curves, left y axis) for a system containing H₂L and Cd(II) (a) Pb(II) (b) or Cu(II) (c) in 1:1 molar ratio. In the case of Cu(II), metal complexation occurs at low pH values and the UV-vis spectra display a band centered at 498 nm, which remains almost unaltered in the pH range 3.5-12.
Figure S5. ESI mass spectra recorded on an aqueous solution containing H₂L and Zn(II) in 1:1 (a) and 1:2 (b) molar ratios.
Figure S6. Fluorescence emission intensity at 525 nm (■, right y axis) as a function of pH compared to the distribution curves of the Cu(II) (a) or Pb(II) (b) complexes (solid curves, left y axis) for systems containing H₂L and Cu(II) or Pb(II) in 1:1 molar ratio ([H₂L] = [Cu(II)] = [Pb(II)] = 2 × 10⁻⁷ M, λ_{exc} 472 nm, 298 K, 0.1 M NMe₄Cl).
**Figure S7.** Flow cytometric evaluation of the intracellular fluorescence distribution in unstained HL60 cells (black line), H$_2$L incubated cells (thick grey line) and H$_2$L incubated, digitonin permeabilized cells (thin grey line). Abscissa: cellular fluorescence intensity displayed on a logarithmic scale, ordinate: number of cells per channel.