A “turn-on”- turning – to - ratiometric sensor for zinc(II) ions in aqueous media

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Supporting Information Available:
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I) Experimental Section

General Procedures: All reactions were carried out under anhydrous conditions in dry solvents, using argon or nitrogen in flame-dried glassware. Reactions were monitored by thin-layer chromatography (TLC) using silica gel plates from Merck (60F254), which were visualized under a UV-Vis Lamp (254 and 366 nm, respectively) or with a 7% ethanolic solution of phosphomolybdic acid. Flash column chromatography was performed in silica gel 60 from Merck (230-400 mesh). NMR spectra were taken on an AMX500 Bruker FT-NMR or a MSL300 Bruker FT-NMR spectrometer; proton chemical shifts are reported in relative to tetramethylsilane. Fluorescence spectra were recorded on an Aminco Bowman spectrofluorimeter (Spectronics Co., USA). All low resolution mass spectra were acquired using sonic-spray ionization (SSI) mass spectrometry (MS). Details of the source construction have been provided and its advantages for the analysis of coordination compounds discussed.¹ In summary, a prototype SSI source (Elemental Scientific, Omaha, USA)
fitted with a modified glass pneumatic nebulizer (TR-30-C1, Meinhard®, Golden, CO, USA) was installed on a ion trap mass spectrometer (LCQ Advantage, Thermo Scientific). SSI operating conditions included a nitrogen gas flow between 50-70 psi and a source induced dissociation voltage between 30 and 50 V, along with a heated ion transfer capillary temperature of 250 °C. HRMS were taken using electrospray ionization orbitrap MS at ProFI, Foundation for Research and Technology-Hellas (ITE), Heraklion, Greece. Ultra-pure water was collected from a PURELAB Ultra instrument by ELGA.

2-(1,3-benzothiazol-2-yl)-4-[bis(2-pyridinylmethyl)amino] phenol, (3):

![Chemical Structure]

To a solution of picolyl chloride hydrochloride (2) (524.5 mg, 3.19 mmol) in 10 ml dry acetonitrile, were added 6 ml DIPEA followed by aminophenol 1 (258.0 mg, 1.07 mmol) and potassium iodide (264.8 mg, 1.59 mmol). The system was heated under reflux overnight. After the completion of the reaction, as indicated by TLC (50% ethyl acetate in petroleum ether), the solution was extracted with water and brine the organic phase was dried over MgSO₄, filtered and the solvent was distilled in high vacuum. The product was isolated through flash liquid gradient chromatography, using 5-70% acetone in toluene as eluent. Chromatography yielded 258 mg of 3 (57%). ¹H NMR (500 MHz, CDCl₃): δ 11.8 (s, 1H), 8.66 (d, J=4.5Hz, 2H), 7.89 (d, J=8.1Hz, 1H), 7.79 (d, J=7.8Hz, 1H), 7.6 (dd, J₁=7.8Hz J₂=1.8Hz, 2H), 7.32 (d, J=7.8Hz, 1H), 7.16 (t, J=6.0Hz, 3H), 6.98 (d, J=3.0Hz, 3H), 6.92 (d, J=9.0Hz, 1H), 6.85 (dd, J₁=9.0Hz J₂=3.0Hz, 1H), 7.31 (m, 1H), 4.79 (m, 4H). ¹³C NMR (300 MHz, CDCl₃): δ: 169.67, 158.91, 158.90, 152.08, 150.38, 149.63, 149.61, 141.55, 141.54, 136.89, 132.74, 126.55, 125.32, 125.31, 122.20, 122.12, 121.45, 121.36, 119.47, 118.56, 116.56, 112.11, 58.52. SSI-MS: 425.53 (M+H)⁺. HRMS: calculated for C₂₅H₂₀N₄OS: 425.1431, found 425.1430.
Trimethylsilyl 2-(1,3-benzothiazol-2-yl)-4-[bis(2-pyridinylmethyl)amino] phenol (4)

The trimethylsilyl ether of phenol 3 was prepared quantitatively in ten minutes, under standard literature conditions.\(^2\) \(^1\)H NMR (500 MHz, CDCl\(_3\)): 8.67 (d, J=4.5Hz, 2H), 8.28 (d, J=8.0Hz, 1H), 7.98 (d, J=3.0Hz, 1H), 7.92 (d, J=8.0 Hz, 1H) 7.50 (dt, J\(_1\)=8.0 Hz J\(_2\)=1.5Hz, 2H), 7.46 (dd, J\(_1\)= J\(_2\)=8.0 Hz, 1H), 7.37-7.34 (m, 3H), 7.19 (dd, J\(_1\)=5.5 Hz J\(_2\)=1.5 Hz, 2H), 6.83 (d, J=9.0Hz, 1H), 6.76 (dd, J\(_1\)=9.0Hz J\(_2\)=3.0Hz, 1H), 4.87 (s, 4H), 0.37 (s, 9H). \(^{13}\)C NMR (300 MHz, CDCl\(_3\)): \(\delta\) 163.75, 158.89, 152.13, 149.48, 145.78, 143.16, 136.95, 136.05, 125.76, 124.71, 12 4.49, 122.82, 122.13, 121.32, 121.20, 120.04, 116.74, 113.16, 57.39, 0.69. HRMS: calculated for C\(_{28}\)H\(_{39}\)N\(_4\)OSSi: 497.1826, found 497.1829.
II) Spectral Data for Compounds 3 and 4.

\(^1\)H NMR spectrum of compound 3 (500 MHz, CDCl\(_3\))
$^{13}$C NMR spectrum of compound 3 (300 MHz, CDCl$_3$)
$^1$H NMR spectrum of compound 4 (500 MHz, CDCl$_3$)
$^{13}$C NMR spectrum of compound 4 (300 MHz, CDCl$_3$)
Zn$^{2+}$ binding to ligand 3 Mr=424.5

SSI-MS Analysis with Type C nebulizer N$_2$ pressure = 50psi

Sonic-spray ionization mass spectrum of the zinc(II)-probe ligand ($M_r$ 424.5) present at 10 μM in methanol.
SSI mass spectrum of solution containing zinc(II)-probe ligand and Zn(ClO\(_4\))\(_2\) at a 1:1 molar ratio (each was at a concentration of 2 \(\mu\)M upon mixing).
SSI-mass spectrum of probe 3 and Zn$^{2+}$ mixed at a 1:10 ratio
Ligand to Zn(ClO$_4$)$_2$ 1:10 molar ratio

SSI mass spectrum of solution containing zinc(II)-probe ligand and Zn(ClO$_4$)$_2$ at a 1:10 molar ratio (ligand was at a concentration of 2 µM, whereas Zn$^{2+}$ was at a concentration of 20 µM).
III) Fluorescence Spectra for 3 in the free and Zn\(^{2+}\)-bound form

Preparation of indicator solutions containing adjusted Zn\(^{2+}\) concentrations\(^3\)

A buffer solution of 50mM HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid), containing 0.1M KNO\(_3\) and 10mM EGTA (ethylenediaminetetraacetic acid) was prepared. The pH was adjusted to 7.2 via addition of KOH solution. In 3ml samples of the above solution were added the appropriate aliquots of 1M ZnSO\(_4\) solutions to give a series of solutions with known concentrations of free Zn\(^{2+}\) according to a published procedure.\(^4\) To each of the above solutions were added 20\(\mu\)l aliquots of a 10mM in DMSO (\(\geq 99.5\%\)) dye solution to make a final indicator concentration of 6.7 \(\mu\)M. All solutions were prepared using nanopure water.
Excitation Spectra of 3 in nanomolar and micromolar Zn$^{2+}$ concentrations

Excitation spectra of dye 3 ($6.7 \times 10^{-5}$M) in solutions of increasing Zn$^{2+}$ concentrations at nanomolar range. The emission wavelength was set at 615 nm. Experiments performed in 10 mM EGTA, 50 mM HEPES, 0.1M KNO$_3$, at RT and pH=7.2

Excitation spectra of dye 3 ($6.7 \times 10^{-5}$M) in solutions of increasing Zn$^{2+}$ concentrations at micromolar range. The emission wavelength was set at 515 nm. Experiments performed in 10 mM EGTA, 50 mM HEPES, 0.1M KNO$_3$, at RT and pH=7.2
VI. DFT geometry optimization for the 3-(Zn$^{2+}$)·2H$_2$O complex

**Fig. S1.** DFT geometry optimization for the 3-(Zn$^{2+}$)·2H$_2$O complex. Atoms are represented in colours; zinc: orange, sulphur: yellow, nitrogen: blue, oxygen: red, carbon: grey, hydrogen atoms are not shown for simplicity.

DFT geometry optimization for the 3-(Zn$^{2+}$) and 3-(Zn$^{2+}$)·2H$_2$O complexes: Alternative binding of the zinc(II) ion on the chromophore moiety

**Fig. S2** DFT geometry optimization for the 3-(Zn$^{2+}$) and 3-(Zn$^{2+}$)·2H$_2$O complexes: Alternative binding of the zinc(II) ion on the chromophore moiety. Atoms are represented in colours; zinc: orange, sulphur: yellow, nitrogen: blue, oxygen: red, carbon: grey, hydrogen atoms are not shown for simplicity.
DFT geometry optimization for the 3-(Zn^{2+})_2 and 3-(Zn^{2+})_2.4H_2O complexes

Fig. S3. DFT geometry optimization for the 3-(Zn^{2+})_2 and 3-(Zn^{2+})_2.4H_2O complexes. Atoms are represented in colours; zinc: orange, sulphur: yellow, nitrogen: blue, oxygen: red, carbon: grey, hydrogen atoms are not shown for simplicity

V. Detection limit of probe 3 for Zn^{2+} ions.

Fig. S4. Fluorescence intensity of the sensor at each concentration of zinc(II) ion, normalized between the minimum fluorescence intensity, found at zero free zinc(II) (shown on the graph as 1.0 nM), and the maximum fluorescence intensity, found at 67.0 nM. A linear regression curve was fitted to the six intermediate values (22.0 nM -54.0 nM) of Fig. S4. The point at which this line crossed the ordinate axis was taken as the detection limit and equalled approximately 5.0 nM.\(^5\)
VI. Titration profiles of 3 based on fluorescence changes and ratiometric calibration changes upon Zn$^{2+}$ binding at nM and mM concentrations, respectively.

Fluorescence Titration of 3 in Solutions of Increasing Zinc(II) Concentration

![Fluorescence Titration Graph](image)

**Fig. S5.** Titration profiles of 3 based on fluorescence changes upon Zn$^{2+}$ binding at nM concentrations.

Fluorescence Ratio Changes of 3 in Solutions of Increasing Zinc(II) Concentrations

![Fluorescence Ratio Graph](image)

**Fig. S6.** Titration profiles of 3 based on ratiometric calibration changes upon Zn$^{2+}$ binding at mM concentrations.
VII. Excitation and emission spectra of 4 in solutions of increasing Zn$^{2+}$ concentrations

**Fig. S7.** Excitation (top) and emission (bottom) spectra of dye 4 ($6.7 \times 10^{-5}$M) in solutions of increasing Zn$^{2+}$ concentrations. The emission and excitation wavelengths were set at 465 nm and 400 nm, respectively. Experiments performed in 10 mM EGTA, 50 mM HEPES, 0.1M KNO$_3$, at RT and pH=7.2
References


