

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

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Supporting Information Available:

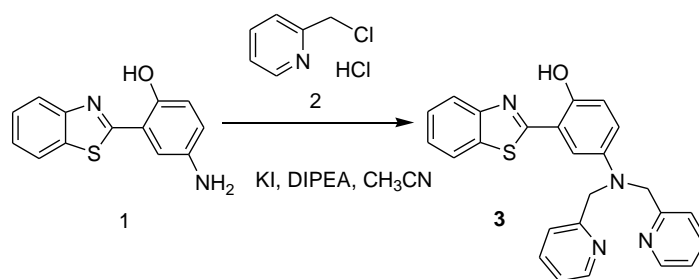
- I) Experimental section
- II) Spectral Data (^1H NMR, ^{13}C NMR of **3** and **4**, and SSI-Mass spectra of **3**)
- III) Fluorescence Spectra for **3** in the free and Zn^{2+} -bound form
- IV) DFT geometry optimization for the **3**- Zn^{2+} and **3**- $(\text{Zn}^{2+})_2$ complexes
- V) Detection limit of probe **3** for Zn^{2+} ions.
- VI) Titration profiles of **3** based on fluorescence changes and ratiometric calibration changes upon Zn^{2+} binding at nM and mM concentrations, respectively.
- VII) Fluorescence Spectra for **4** in the free and Zn^{2+} -bound form

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)

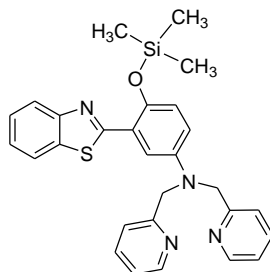
fitted with a modified glass pneumatic nebulizer (TR-30-C1, Meinhard®, Golden, CO, USA) was installed on a ion trap mass spectrometer (LCQ Advantage, Thermo Scientific). SSI operating conditions included a nitrogen gas flow between 50-70 psi and a source induced dissociation voltage between 30 and 50 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):



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 (s, 4H). ¹³C NMR (300 MHz, CDCl₃): δ): 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.

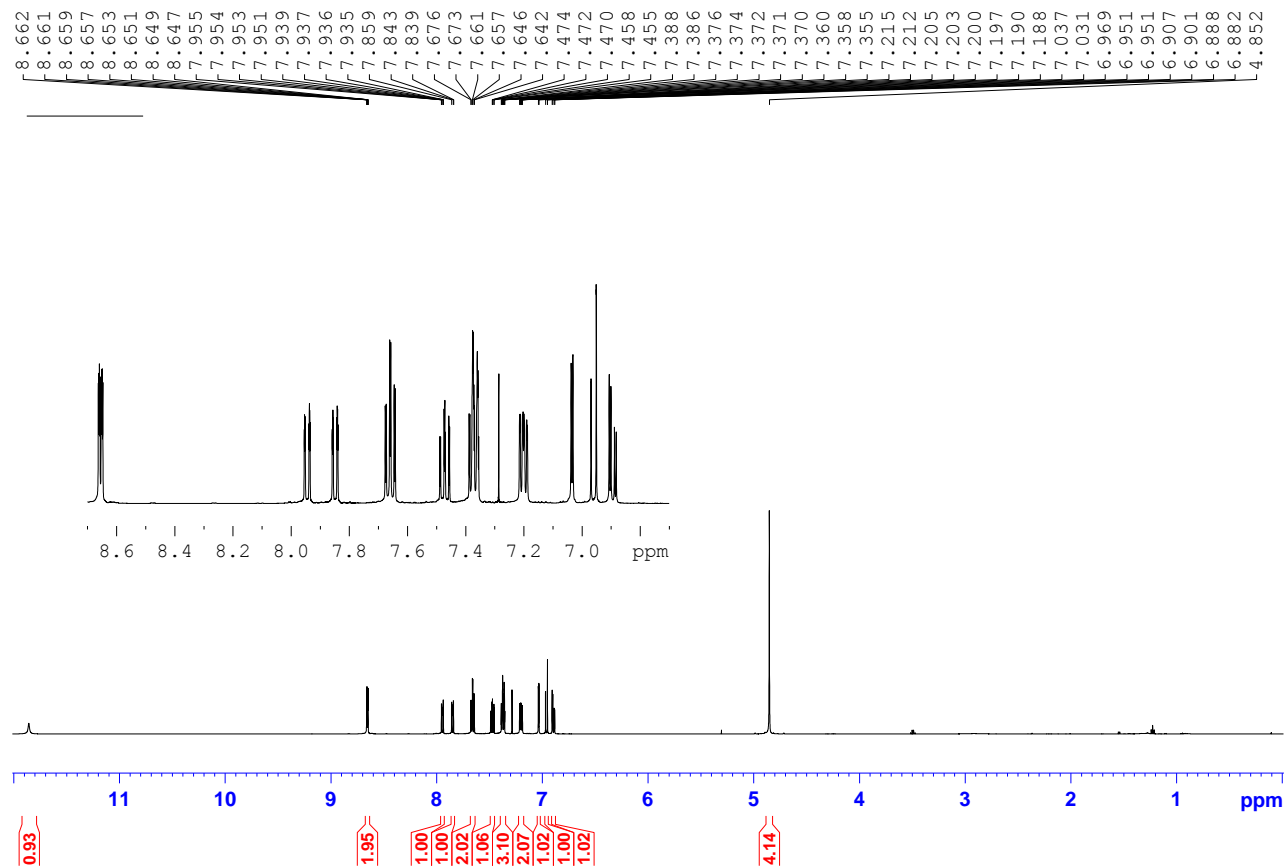
**Trimethylsilyl 2-(1,3-benzothiazol-2-yl)-4-[bis(2-pyridinylmethyl)amino] phenol
(4)**



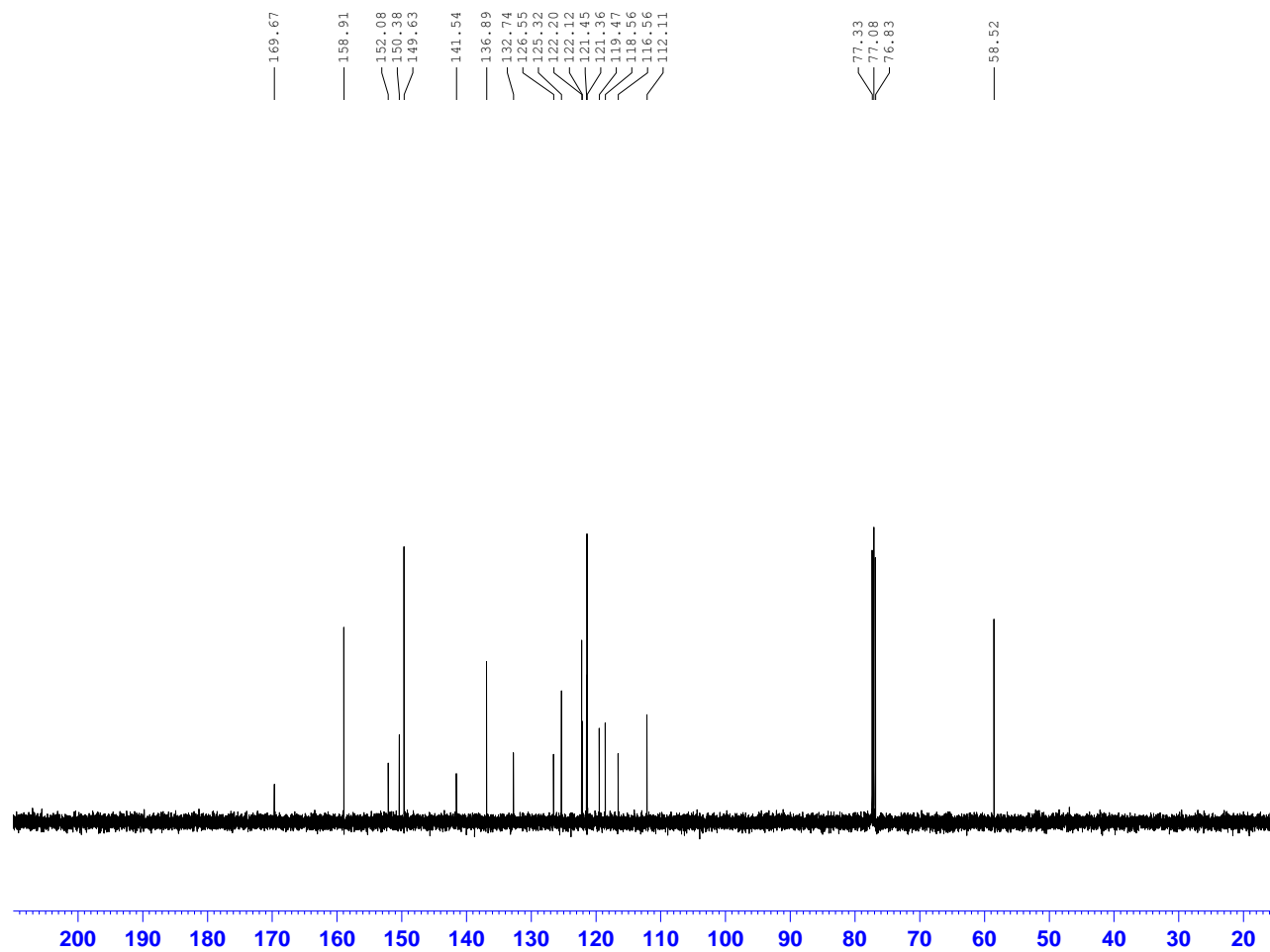
The trimethylsilyl ether of phenol **3** was prepared quantitatively in ten minutes, under standard literature conditions.² ¹H NMR (500 MHz, CDCl₃): 8.67 (d, J=4.5Hz, 2H), 8.28 (d, J=8.0Hz, 1H), 7.98 (d, J=3.0Hz, 1H), 7.92 (d, J=8.0 Hz, 1H) 7.50 (dt, J₁=8.0 Hz J₂=1.5Hz, 2H), 7.46 (dd, J₁= J₂=8.0 Hz, 1H), 7.37-7.34 (m, 3H), 7.19 (dd, J₁=5,5 Hz J₂=1.5 Hz, 2H), 6.83 (d, J=9.0Hz, 1H), 6.76 (dd, J₁=9.0Hz J₂=3.0Hz, 1H), 4.87 (s, 4H), 0.37 (s, 9H). ¹³C NMR (300 MHz, CDCl₃): δ 163.75, 158.89, 152.13, 149.48, 145.78, 143.16, 136.95, 136.05, 125.76, 124.71, 124.49, 122.82, 122.13, 121.32, 121.20, 120.04, 116.74, 113.16, 57.39, 0.69. HRMS: calculated for C₂₈H₂₉N₄OSSi: 497.1826, found 497.1829.

II) Spectral Data for Compounds 3 and 4.

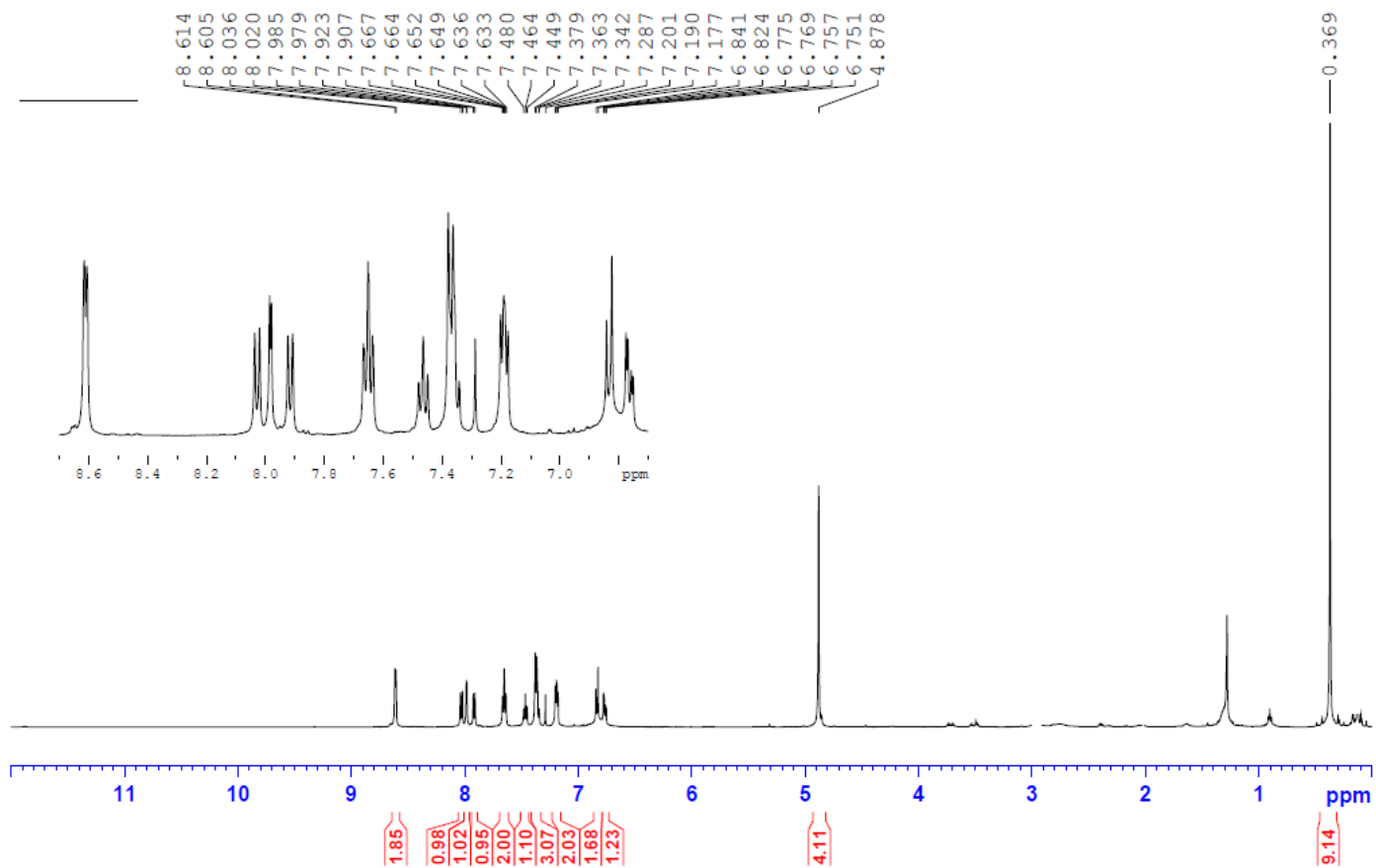
^1H NMR spectrum of compound 3 (500 MHz, CDCl_3)



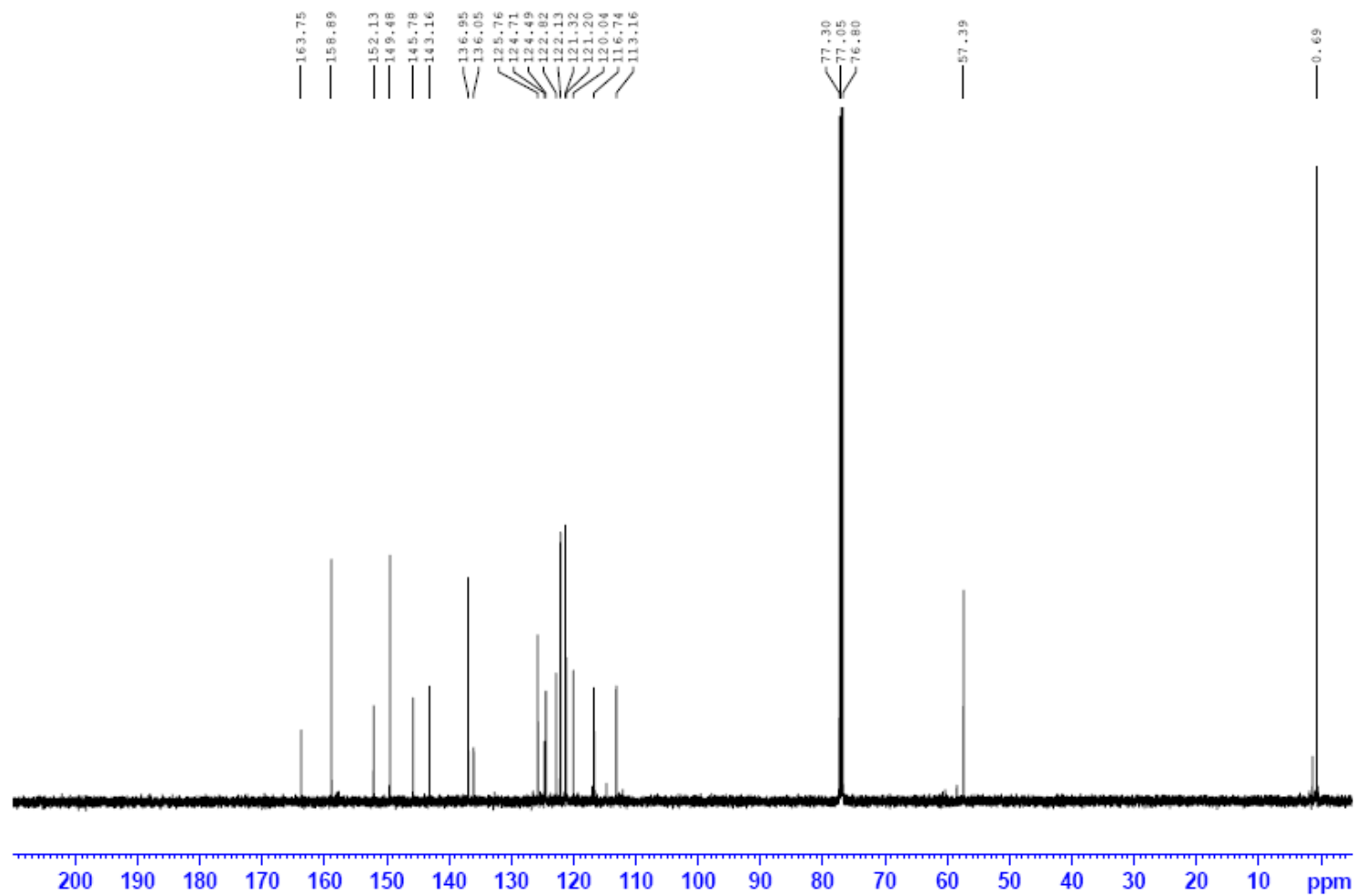
^{13}C NMR spectrum of compound 3 (300 MHz, CDCl_3)



¹H NMR spectrum of compound 4 (500 MHz, CDCl₃)

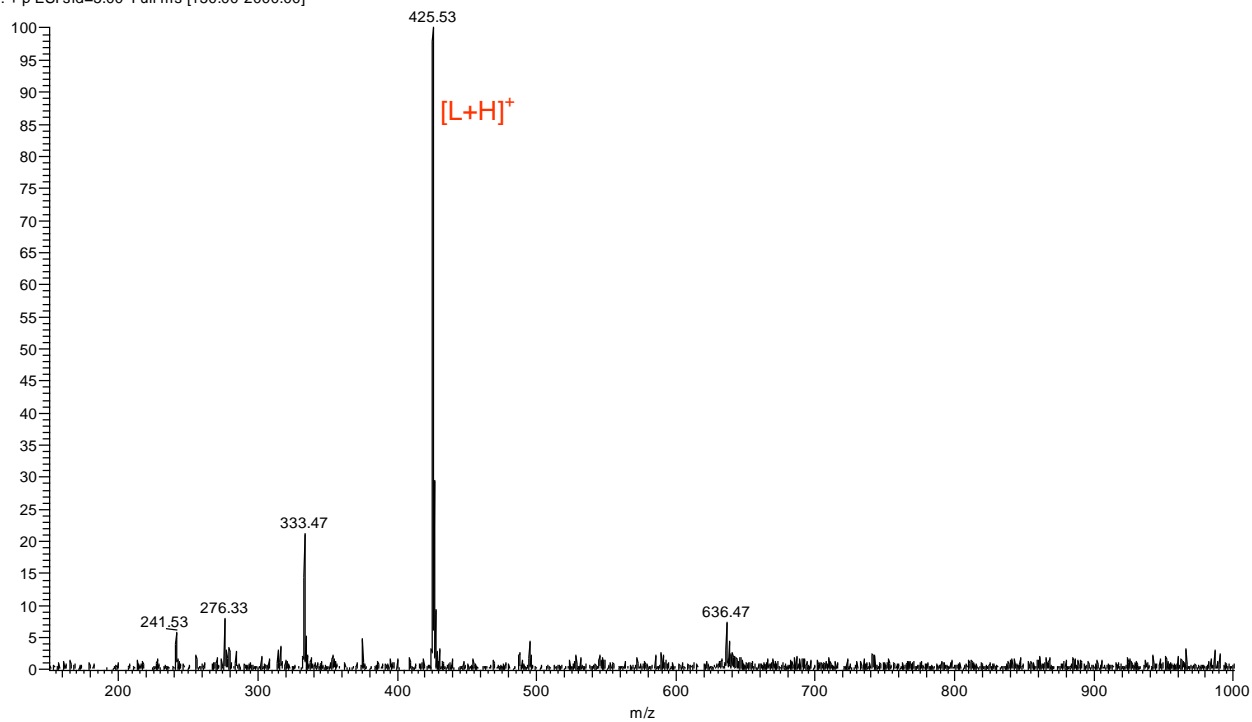


^{13}C NMR spectrum of compound 4 (300 MHz, CDCl_3)



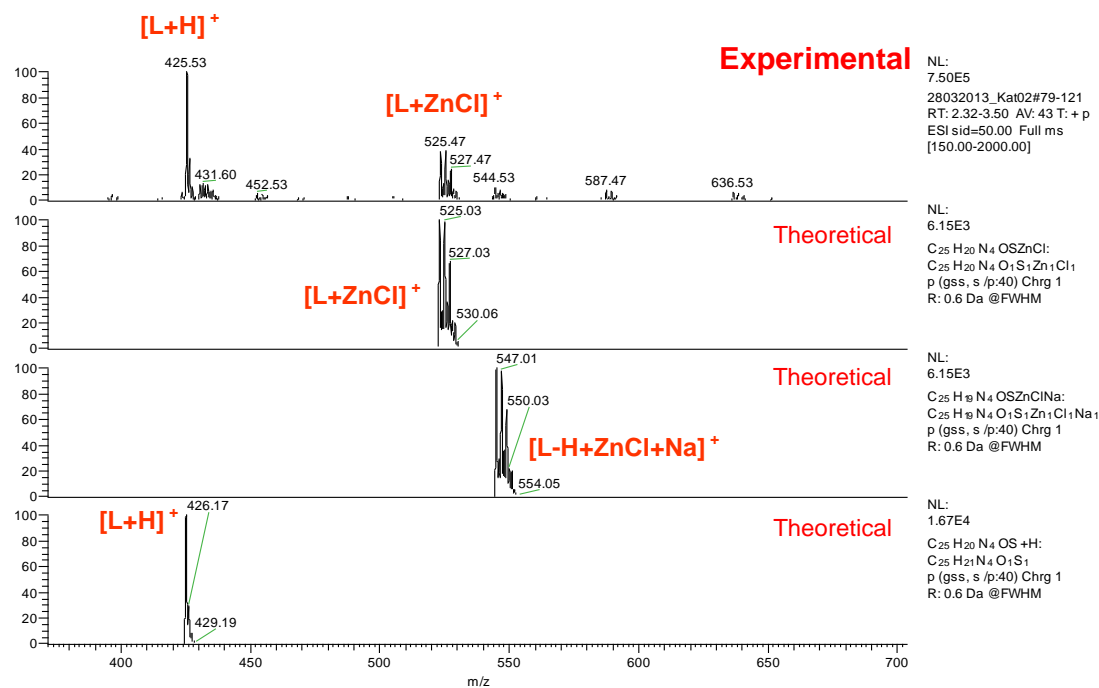
Zn²⁺ binding to ligand 3 Mr=424.5
SSI-MS Analysis with Type C nebulizer N₂ pressure = 50psi

28032013_Kat01 #112-132 RT: 3.14-3.69 AV: 21 NL: 2.39E6
T: + p ESI sid=5.00 Full ms [150.00-2000.00]



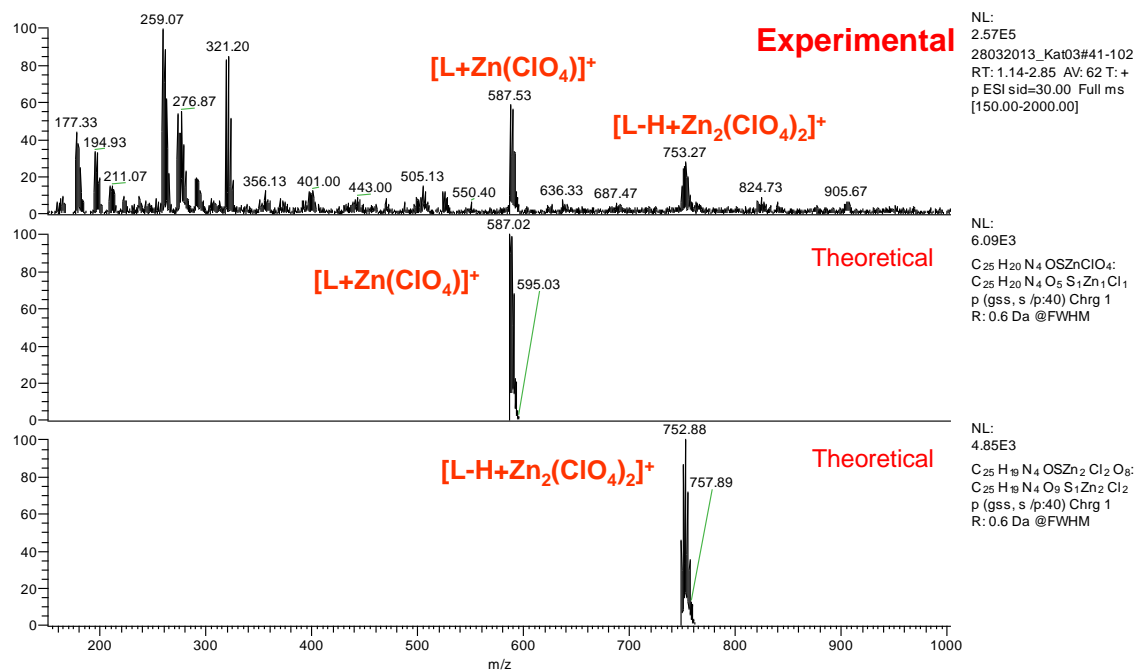
Sonic-spray ionization mass spectrum of the zinc(II)-probe ligand (M_r 424.5) present at 10 μ M in methanol.

SSI-Mass spectrum of probe 3 and Zn^{2+} mixed at equal concentrations Ligand to $Zn(ClO_4)_2$ 1:1 molar ratio



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 μ M upon mixing).

SSI-Mass spectrum of probe 3 and Zn²⁺ mixed at a 1:10 ratio
Ligand to Zn(ClO₄)₂ 1:10 molar ratio



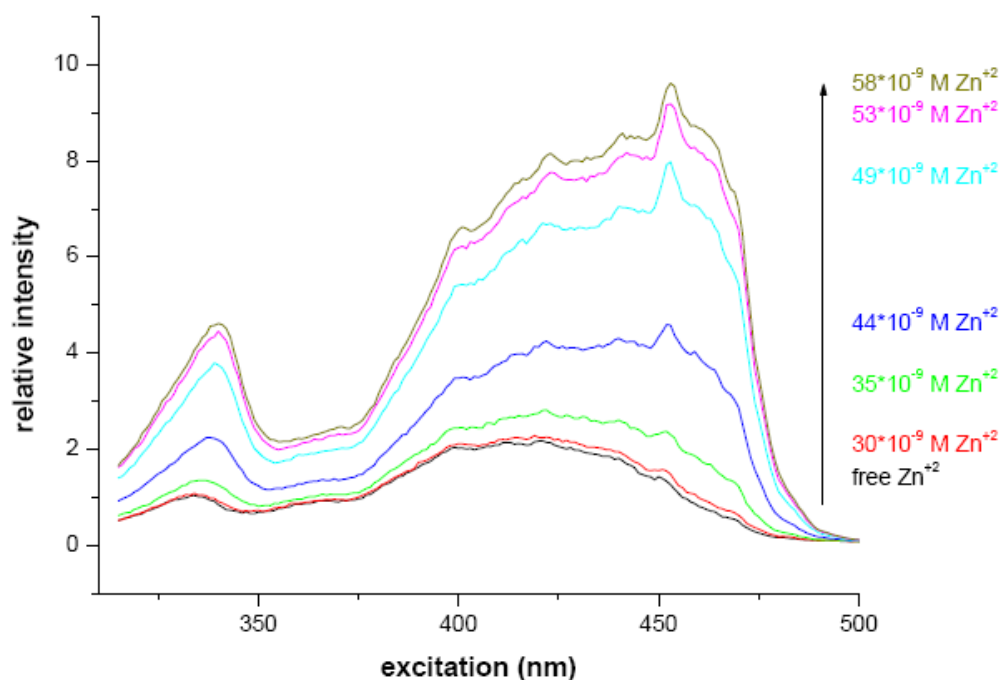
SSI mass spectrum of solution containing zinc(II)-probe ligand and Zn(ClO₄)₂ at a 1:10 molar ratio (ligand was at a concentration of 2 μM, whereas Zn²⁺ was at a concentration of 20 μM).

III) Fluorescence Spectra for 3 in the free and Zn²⁺-bound form

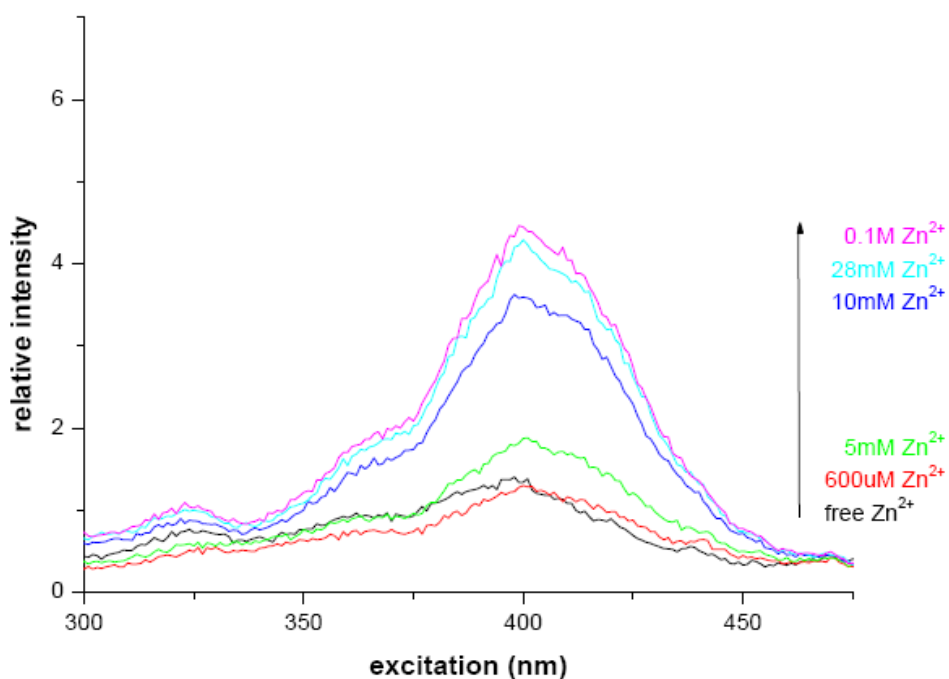
Preparation of indicator solutions containing adjusted Zn²⁺ concentrations³

A buffer solution of 50mM HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid), containing 0.1M KNO₃ and 10mM EGTA (ethylenebis(oxyethylenitrilo) tetraacetic 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 ZnSO₄ solutions to give a series of solutions with known concentrations of free Zn²⁺ according to a published procedure.⁴ To each of the above solutions were added 20μl aliquots of a 10mM in DMSO (≥99.5%) dye solution to make a final indicator concentration of 6.7 μM. All solutions were prepared using nanopure water.

Excitation Spectra of **3** in nanomolar and micromolar Zn^{+2} concentrations



Excitation spectra of dye **3** (6.7×10^{-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** (6.7×10^{-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 $3-(\text{Zn}^{2+})\cdot 2\text{H}_2\text{O}$ complex

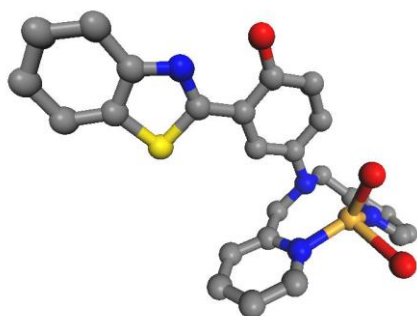


Fig. S1. DFT geometry optimization for the $3-(\text{Zn}^{2+})\cdot 2\text{H}_2\text{O}$ complex. Atoms are represented in colours; zinc: orange, sulphur: yellow, nitrogen: blue, oxygen: red, carbon: grey, hydrogen atoms are not shown for simplicity

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

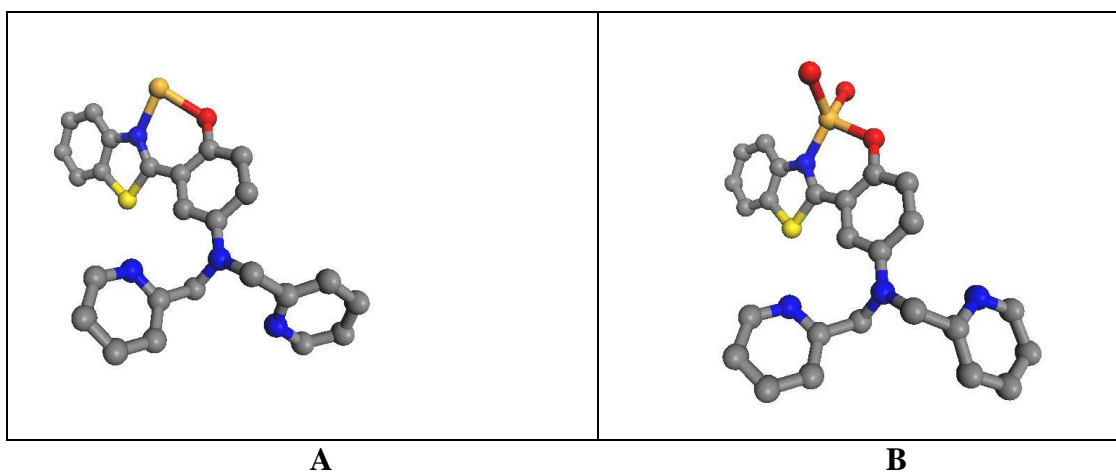


Fig. S2 DFT geometry optimization for the $3-(\text{Zn}^{2+})$ and $3-(\text{Zn}^{2+})\cdot 2\text{H}_2\text{O}$ complexes: Alternative binding of the zinc(II) ion on the chromophore moiety. Atoms are represented in colours; zinc: orange, sulphur: yellow, nitrogen: blue, oxygen: red, carbon: grey, hydrogen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

DFT geometry optimization for the $3-(\text{Zn}^{2+})_2$ and $3-(\text{Zn}^{2+})_2 \cdot 4\text{H}_2\text{O}$ complexes

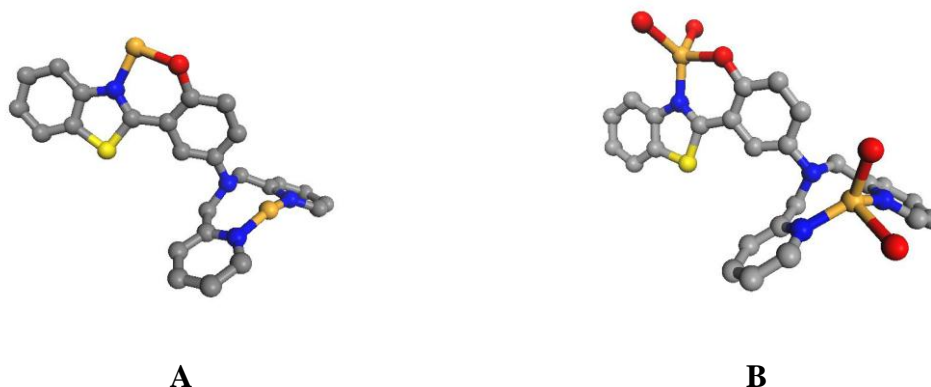


Fig. S3. DFT geometry optimization for the $3-(\text{Zn}^{2+})_2$ and $3-(\text{Zn}^{2+})_2 \cdot 4\text{H}_2\text{O}$ complexes. Atoms are represented in colours; zinc: orange, sulphur: yellow, nitrogen: blue, oxygen: red, carbon: grey, hydrogen atoms are not shown for simplicity

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

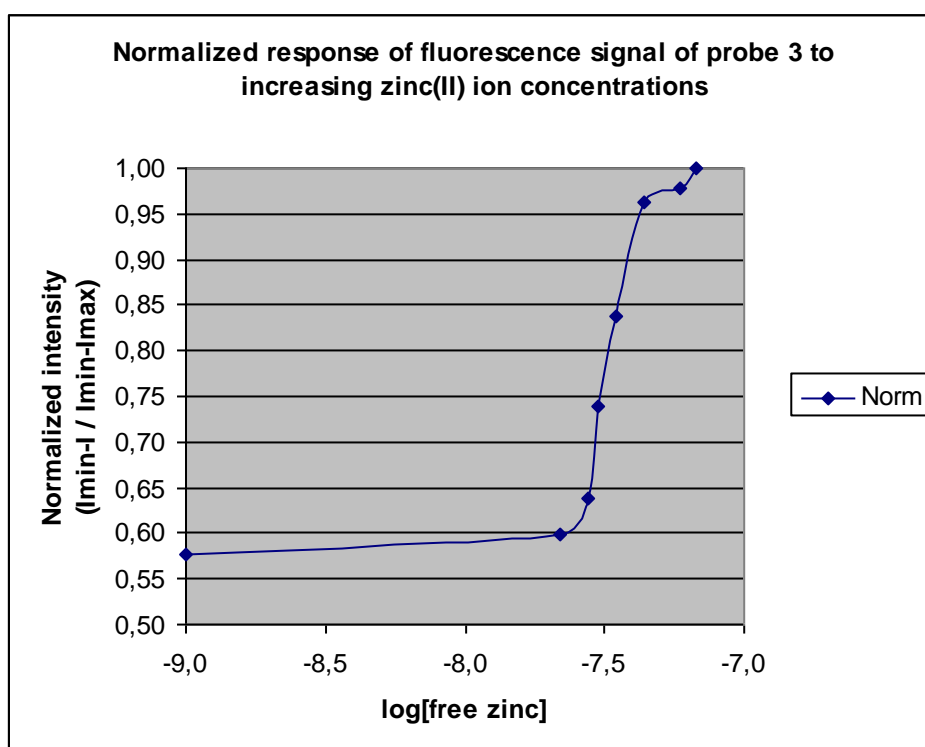


Fig. S4. Fluorescence intensity of the sensor at each concentration of zinc(II) ion, normalized between the minimum fluorescence intensity, found at zero free zinc(II) (shown on the graph as 1.0 nM), and the maximum fluorescence intensity, found at 67.0 nM. A linear regression curve was fitted to the 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

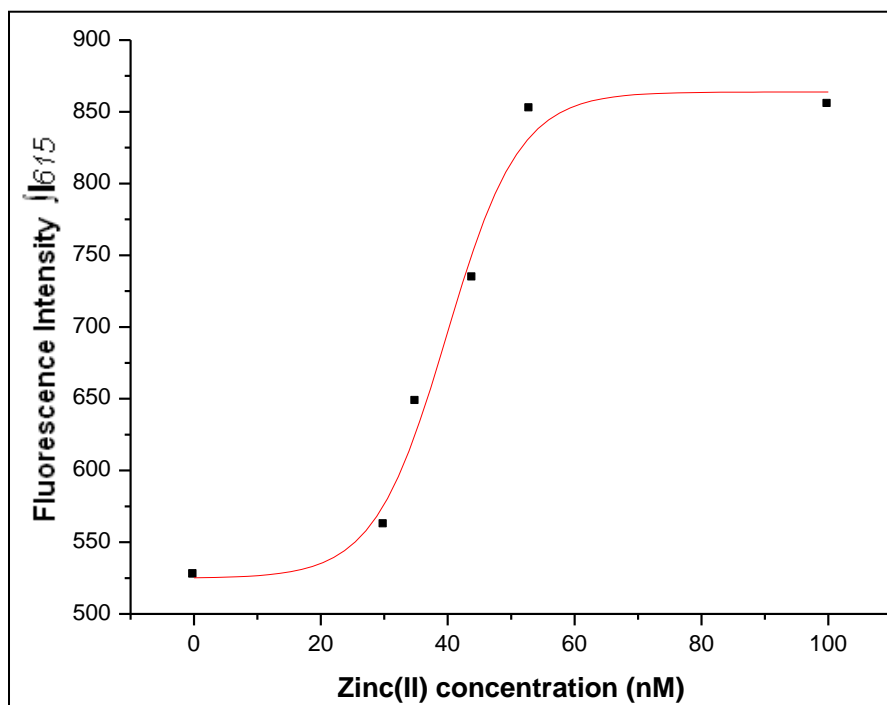


Fig. S5. Titration profiles of **3** based on fluorescence changes upon Zn^{2+} binding at nM concentrations.

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

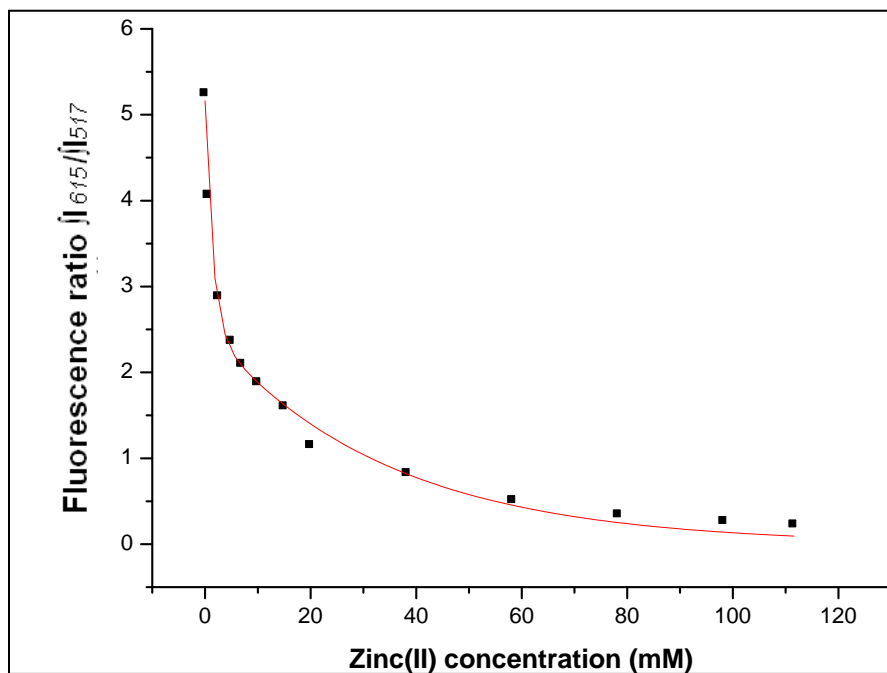


Fig. S6. Titration profiles of **3** based on ratiometric calibration changes upon Zn^{2+} binding at mM concentrations.

VII. Excitation and emission spectra of **4** in solutions of increasing Zn^{2+} concentrations

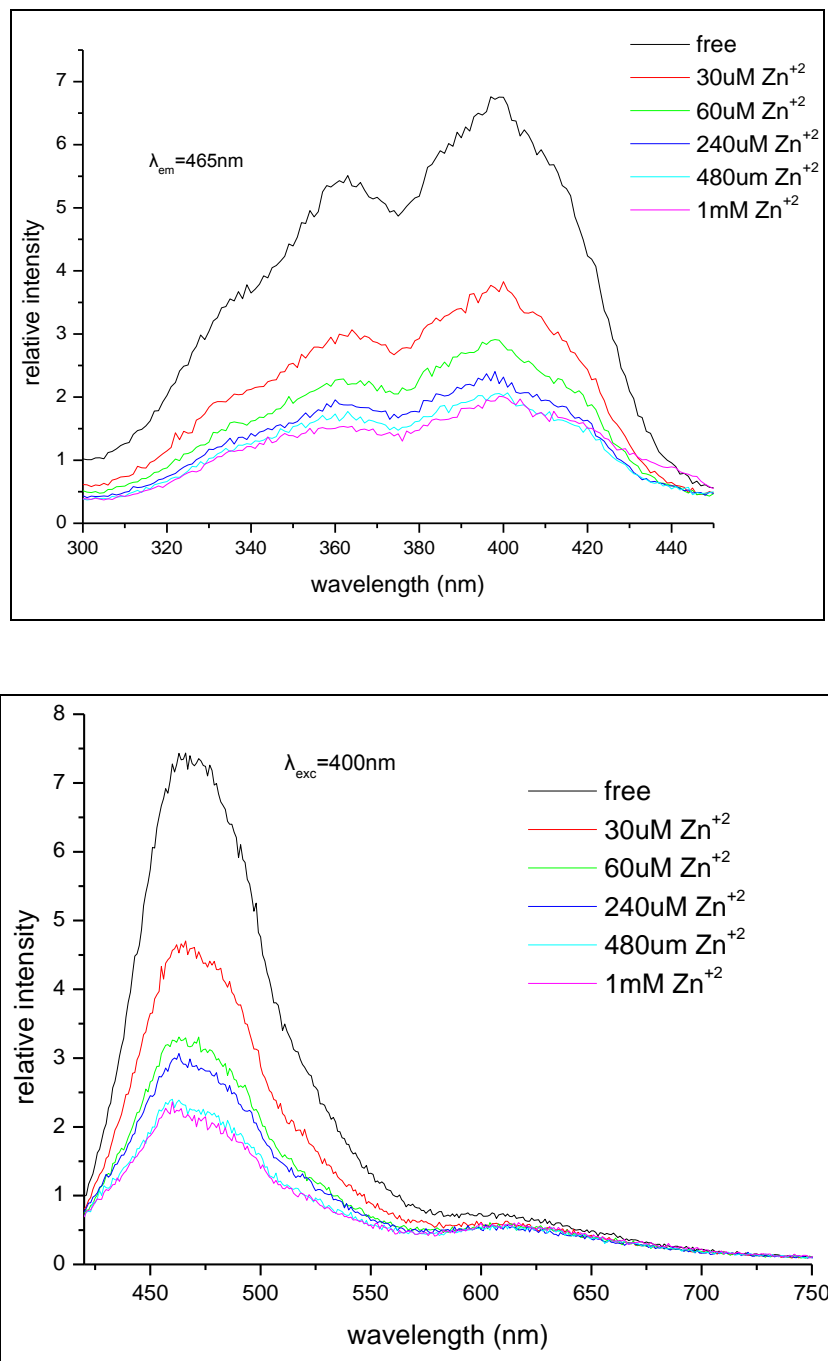


Fig. S7. Excitation (top) and emission (bottom) spectra of dye **4** ($6.7 \times 10^{-5}\text{M}$) in solutions of increasing Zn^{2+} concentrations. The emission and excitation wavelengths were set at 465 nm and 400 nm, respectively. Experiments performed in 10 mM EGTA, 50 mM HEPES, 0,1M KNO_3 , at RT and pH=7.2

References

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- ² E. J. Corey and B. B. Snider, *J. Org. Chem.*, 1973, **38** 3632.
- ³ E. Roussakis, S. Voutsadaki, E. Pinakoulaki, D. P. Sideris, K. Tokatlidis and H. E. Katerinopoulos, *Cell Calcium*, 2008, **44**, 270
- ⁴ K.R. Gee, Z.-L. Zhou, D. Ton-That, S.L. Sensi, and J.H. Weiss, *Cell Calcium*, 2002, **31**, 245.
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