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Highly selective BODIPY-based fluorescent probe for Zn^{2+} imaging in plant roots

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Materials, methods and instrumentations

All chemicals and reagents were commercial and used as received unless otherwise noted. Toluene, acetonitrile (MeCN), dichloromethane (CH_2Cl_2), and tetrahydrofuran (THF) were dried and purified under nitrogen using standard method [S1] and were distilled immediately before use. The structures of synthesized compounds were established using 1D NMR (^1H, ^{13}C) spectroscopy. NMR spectra were acquired on a Bruker Avance 300 spectrometer at room temperature; the chemical shifts δ were measured in ppm relative to the solvent (^1H: $CDCl_3$, δ = 7.27 ppm; ^{13}C: $CDCl_3$, δ = 77.00 ppm). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. The coupling constants (J) are in Hertz. High-resolution and accurate mass spectra were obtained on BrukermicrOTOF-QTM ESI-TOF (Electrospray Ionization/Time of Flight) and Thermo Scientific* LTQ Orbitrap mass spectrometers. EI mass spectra were recorded on a Finnigan MAT Incos 50 spectrometer (70 eV). The melting points (mp) were measured on a Boetius capillary melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was carried out on silica gel plates (silica gel 60 F254 aluminum supported plates); the visualization was accomplished with an UV lamp (365 nm). Column chromatography was performed on neutral aluminum oxide (Brockmann II, pH 9-10) or silica gel 60 (230-400 mesh). UV/Vis absorption spectra and steady-state fluorescence emission spectra were obtained with a Panorama instrument at the IOC RAS using .3-mL quartz cuvettes with a 1 cm path length. The measurements were performed in the presence of air in acetonitrile.

Synthesis of the ZS-1 probe

Compound **ZS-1** was prepared by modified procedures in six preparative steps.

Ethyl 4-acetyl-3,5-dimethyl-1H-pyrrole-2-carboxylate. To a solution of ethyl acetoacetate (128 mL, 1.0 mol) in acetic acid (80%, 250 mL), a cold solution of sodium nitrite (75 g, 1.1 mol) in H_2O (250 mL) was added at 0 °C with stirring. The resulting mixture was stirred for 12 h at 25 °C and then cooled to 0 °C. Acetylacetone (102 mL, 1.0 mol) and zinc dust (130 g, 2.0 mol) were added. The mixture was stirred at 60 °C for 2 h, cooled to ~ 20 °C and extracted with CH_2Cl_2 (3×300 mL). The extract was concentrated *in vacuo* followed by crystallization from $CHCl_3$ to obtain the title product as a white solid, yield: 90 g (43%), mp = 140 °C (lit. mp = 142 °C [S2]). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.34 (t, *J* = 7.1 Hz, 3H), 2.42 (s, 3H), 2.51 (s, 3H), 2.57 (s, 3H), 4.34 (q, *J* = 7.1 Hz, 2H), 9.41 (br.s, 1H, NH); MS (EI) m/z: 209 [M^+].

3-Acetyl-2,4-dimethyl-1H-pyrrole. A mixture of the above compound (90 g, 0.43 mol) and KOH (48 g, 2.0 mol) in ethylene glycol (300 mL) was heated at 160 °C for 4 h. After cooling, the mixture was diluted with water (300 mL) and extracted with $CHCl_3$ (6×100 mL). The extract was washed with brine and dried over Na_2SO_4. The solvent was removed to obtain a white solid, yield: 42 g (74%), mp = 96 °C (lit. mp = 96 °C [S3]); ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.26 (s, 3H), 2.43 (s, 3H), 2.49 (s, 3H), 6.36 (s, 1H), 8.52 (br.s 1H, NH); MS (EI) m/z: 137 [M^+]

3-Ethyl-2,4-dimethyl-1H-pyrrole **1** [S4]. To a stirred suspension of $LiAlH_4$ (15.2 g, 0.4 mmol) in dry THF (160 mL), the above compound (42 g, 0.32 mol) in THF (400 mL) was added. The mixture was refluxed for 10 h and cooled to room temperature. The excess hydride was decomposed with aqueous saturated Na_2SO_4. The mixture was filtered, and the filtrate was extracted with $CHCl_3$ (3×200 mL). The extract was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was distilled under reduced pressure to give a colorless liquid, yield: 24.5 g (65%), bp = 85-88 °C at 760 Torr (lit. bp = 84-87 °C [S4]). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.20 (t, 3H, J = 8.1 Hz), 2.15 (s, 3H), 2.27 (s, 3H), 2.52 (q, J = 8.1 Hz, 2H), 6.47 (s, 1H), 7.51 (br.s, 1H, NH); MS (EI) m/z: 123 [M^+].

8-Chloromethyl-2,6-diethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene **2** [S5]. To a solution of compound **1** (0.85 mL, 6.3 mmol) in dry CH_2Cl_2 (20 mL), chloroacetyl chloride (0.25 mL, 3.1 mmol) was added dropwise under inert atmosphere with stirring. The resulting mixture was refluxed for 1 h and cooled to room temperature. The solvent was removed under reduced pressure. The residue was re-dissolved in a toluene/CH_2Cl_2 mixture (40 mL/2 mL), and then Et_3N (1.7 mL, 12.2.

mmol) was added in one portion. The mixture was stirred at 25 °C for 15 min followed by the addition of $BF_3 \cdot Et_2O$ (1.9 mL, 15.7 mmol). The resulting mixture was stirred at 50 °C for 1 h, cooled to room temperature and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 (30 mL). The organic fraction was washed with water (3×10 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography (Al_2O_3, eluent petroleum ether/CH_2Cl_2, 1 : 1) to obtain purple-green solid, yield: 0.25 g (23%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.02 (t, 6H, J = 7.3 Hz, $CH_2C\underline{H}_3$), 2.40 (q, 4H, J = 7.3 Hz), 2.45 (s, 6H), 2.50 (s, 6H), 4.97 (s, 2H, CH_2Cl). HRMS (ESI): Calculated for $C_{18}H_{24}BClF_2N_2Na$ $(M+Na)^+$ 375.1585, found 375.1584.

8-[N,N-Di(2-picolyl)aminomethyl]-2,6-diethyl-4,4-difluoro-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene ***ZS-1*** [S6]. To a solution of compound **2** (80 mg, 0.2 mmol) in dry MeCN (20 mL), potassium iodide (73.0 mg, 0.44 mmol), K_2CO_3 (61 mg, 0.44 mmol) and di(2-picolyl)amine (80 mg, 0.40 mmol) were added with stirring. The resulting was refluxed for 8 h and cooled to room temperature. The solvent was removed *in vacuo*. The residue was re-dissolved in H_2O (50 mL), and the water fraction was extracted with CH_2Cl_2 (3×20 mL). The organic fraction was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography (Al_2O_3, CH_2Cl_2) to obtain purple solid (3.4 mg, 10%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.04 (t, 6H, J = 7.3 Hz, $CH_2C\underline{H}_3$), 2.25 (s, 6H), 2.40 (q, 4H, J = 7.3 Hz, $\underline{CH_2}CH_3$), 2.49 (s, 6H), 3.93 (s, 4H), 4.25 (s, 2H), 7.17-7.32 (m, 4H), 7.71 (dd, 2H, J = 1.4, 8.1 Hz), 8.55 (d, 2H, J = 5.1 Hz). ^{13}C NMR ($CDCl_3$, 300 MHz): 12.5, 13.8, 14.8, 17.2, 29.7, 45.8, 49.0, 57.3, 99.8, 103.9, 122.4, 124.4, 132.4, 133.3, 136.9, 137.4, 148.4, 153.5, 161.0, 165.3, 184.9, 186.1. HRMS (ESI): Calculated for $C_{30}H_{36}BFN_5$ $(M-F)^+$ 496.3048, found 496.3040.

Quantum yield measurements

Three different measurements (in different solutions) were performed for each quantum yield. The sample concentrations were chosen to obtain an absorbance of *ca*. 0.01. The relative quantum efficiencies were determined by comparing the areas under the corrected emission spectrum. Quinine sulfate (Φ_F = 0.55 in 0.1M H_2SO_4) was used as the standard. In all Φ_F determinations, the correction for the solvent refractive index (η) was applied. Measurements were performed in spectrophotometric grade CH_3CN/H_2O (1:3 ratio). The equation $\Phi_x = \Phi_{st} (I_x/I_{st}) (A_{st}/A_x) (\eta_x^2/\eta_{st}^2)$ was used to calculate the quantum yield of the sample, where Φ_{st} is the reported quantum yield of the standard, I is the integrated emission spectrum on the cm^{-1} scale, A is the absorbance at the excitation wavelength and η is the refractive index of the solvents used; the x subscript denotes unknown and st denotes standard.

Root incubation and imaging

Roots of 21-day-old canola plants were washed with a 10 mM EDTA solution, after which several adventitious roots from each plant were cut off approximately 40 mm from the apex and incubated in a 4 μM solution of the **ZS-1** dye in 10 mM MES buffer (pH 5.8) at room temperature in the dark for 1 h. In comparative experiments, roots were stained in a 20 μM solution of the commercially available zinc sensor Newport Green™ DCF diacetate (N7991, Thermo Fisher Scientific, USA) in 10 mM MES buffer for 1.5 h. After staining, the roots were washed in the same buffer for 10 min, mounted on slides and photographed with a microscope. Fluorescence microscopy was performed using an AxioImager Z2 microscope (Zeiss AG, Germany) equipped with an N-Achroplan 5x/0.13 Ph0 objective and an appropriate set of excitation/emission filters. The fluorescence intensity was measured continuously from the root apex and up to 25 mm from the apex.
The semiquantitative analysis of the fluorescence intensity in the micrographs was performed with the public domain software ImageJ v.1.49 (NIH; https://imagej.nih.gov/ij/). The autofluorescence intensity was subtracted from the total fluorescence to obtain the dye fluorescence level.

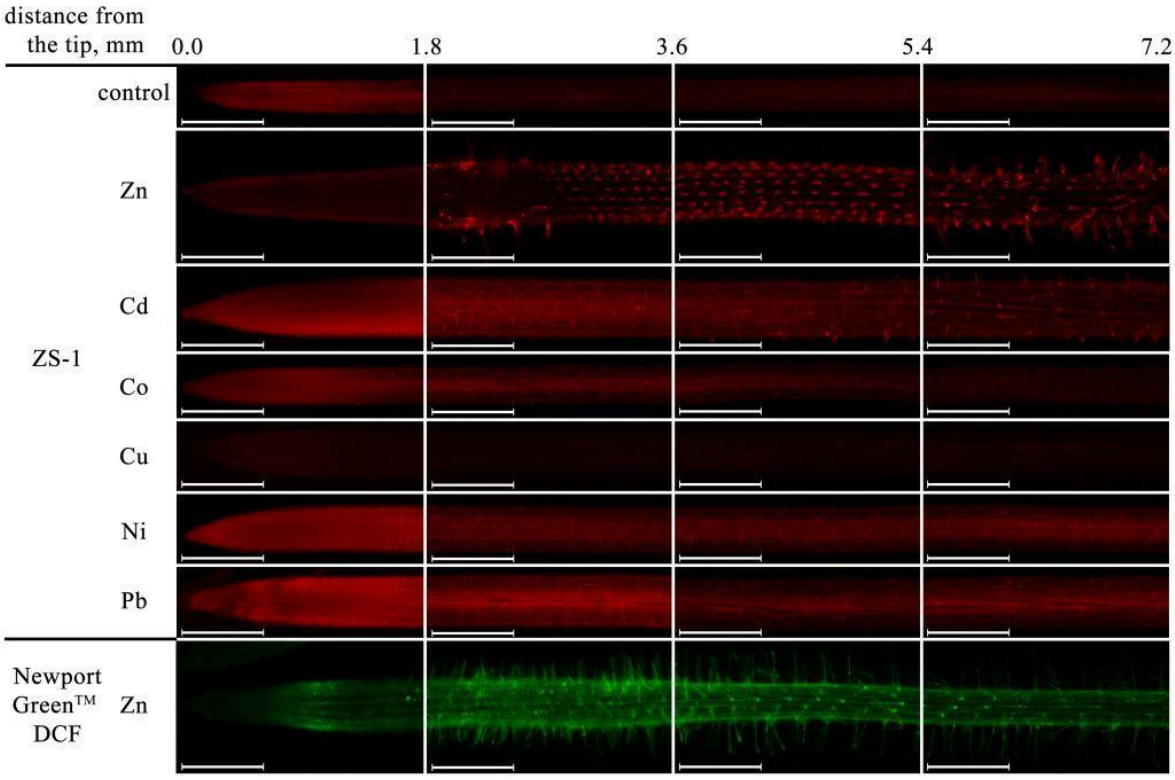

Figure S1. *Upper part*: bioimaging of *Brassica napus L*. roots treated with different metals with **ZS-1**. Cd, Cu and Pb were in 50 μM, Zn, Co and Ni – 250 μM for 3 days. *Lower part*: bioimaging of *Brassica napus L*. roots treated with zinc excess with Newport Green™ DCF sensor. Scale bars represent 500 μm.

References

[S1] W. L. F. Armarego and C. Chai, *Purification of Laboratory Chemicals*, 7th edn., Butterworth-Heinemann, London, 2012.

[S2] E. V. Antina, G. B. Guseva, A. E. Loginova, A. S. Semeikin and A. I. V'yugin, *Russ. J. Gen. Chem.*, 2010, **80**, 2374 (*Zh. Obshch. Khim.*, 2010, **80**, 1911).

[S3] S. Mula, A. K. Ray, M. Banerjee, T. Chaudhuri, K. Dasgupta and S. Chattopadhyay, *J. Org. Chem.*, 2008, **73**, 2146.

[S4] W. Johnson, E. Markham, R. Price and K. B. Shaw, *J. Chem. Soc.*, 1958, 4254.

[S5] L. Zeng, E. W. Miller, A. Pralle, E. Y. Isacoff and C. J. Chang, *J. Am. Chem. Soc.*, 2006, **128**, 10.

[S6] R. Guliyev, O. Buyukcakir, F. Sozmen and O. A. Bozdemir, *Tetrahedron Lett.*, 2009, **50**, 5139.