

Highly selective BODIPY-based fluorescent probe for Zn²⁺ imaging in plant roots

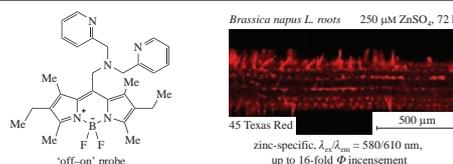
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DOI: 10.1016/j.mencom.2018.11.017

A highly sensitive and selective BODIPY-based ‘off-on’ chemosensor for efficient Zn²⁺ imaging in biological systems has been elaborated.



Zinc is one of the most abundant elements in the Earth's crust and is the second, after iron, most abundant transition metal in plants.¹ Zinc is easily absorbed and accumulated in plant tissues where the root is the primary site of accumulation.² This metal plays an important role in processes associated with plant development, such as root growth and leaf expansion.³ Meanwhile, zinc is toxic for plants at high concentrations, therefore it is necessary to monitor its accumulation by plants.^{4–8}

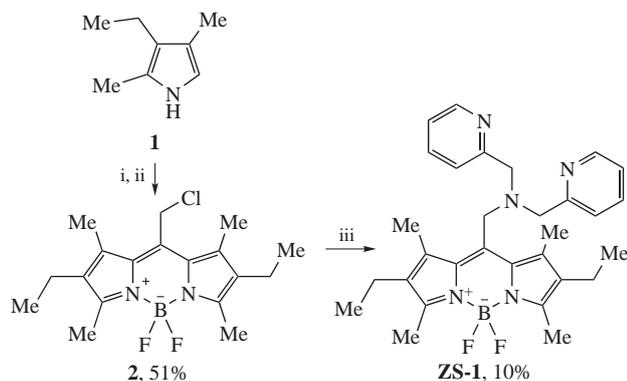
The evaluation of the intracellular labile zinc pool in plant tissues is still a great challenge.⁹ The fluorescence detection stands out as the most effective means to monitor the intracellular Zn²⁺ in biological systems because zinc is spectroscopically and magnetically undetectable.¹⁰ Much effort has been made to develop efficient fluorescent chemosensors for the detection of Zn²⁺ in plant tissues over the past few years.^{11–14} Fluorescent di(2-picolyl)amine (DPA) derivatives were proved to be high-potential probes.^{15–18} On the other hand, among a variety of fluorophores, 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) derivatives are of special interest during past decades due to their remarkable properties.^{19–21} However, to the best of our knowledge, the BODIPY-based chemosensors still were not used to monitor heavy metals, including zinc, in plant tissues. Recently, it was demonstrated that DPA chelator linked through a methylene linker to BODIPY core could be applied for zinc cation imaging *in vitro* in cancer cells.²²

Here we report on the extension of the cited work by designing an efficient *in vivo* zinc(II)-specific fluorescent probe **ZS-1**. We improved this previously reported probe²² by introducing ethyl groups at 2- and 6-positions of the BODIPY core, which caused amplification of spectral characteristics and made it applicable for plant imaging.

The direct synthesis of **ZS-1** was achieved by a method similar to one published previously for the synthesis of BODIPY derivatives²² (Scheme 1). First, condensation of 3-ethyl-2,4-dimethyl-1*H*-pyrrole **1** with chloroacetyl chloride followed by complexation with BF₃·OEt₂ in the same reactor resulted in 8-chloromethyl-substituted BODIPY **2**. Replacement of chlorine in compound **2** with di(2-picolyl)amine under mild conditions afforded product **ZS-1** in 10% yield (for experimental details, see Online Supplementary Materials).

At first, **ZS-1** was examined by absorption and steady-state fluorescence spectroscopy in MeCN solutions. The principal result is that **ZS-1** exhibits a typical BODIPY-like absorption profile with a strong absorption band at 538 nm and a weaker absorption band at 506 nm. The **ZS-1** probe has a high molar absorption coefficient ($\epsilon = 2.2 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$). The fluorescence spectra of **ZS-1** display a broad emission band at 548 nm and show good symmetry to absorption band. The FWHM (full width at the half-maximum height) is 373 nm. The quantum yield of the bound free sensor is low (0.06 in MeCN/H₂O, 1:3) due to photoinduced electron transfer (PET) quenching of the excited state of the boradiaza-indacene moiety (electron acceptor) by the lone pair on the nitrogen atoms (electron donor) of the **ZS-1** moiety.

The addition of Zn²⁺ to **ZS-1** solutions resulted in strong changes in the absorption and fluorescence emission spectra (Figure 1). The absorption maximum of the **ZS-1** complex with Zn²⁺ in MeCN exhibits a strong red shift to 556 nm relevant to a change in the colour of the solution to pink-purple [Figure 1(a)]. The molar absorption coefficient changes only slightly from 2.2×10^4 to $1.6 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ upon Zn²⁺ binding. A slight weakening of the absorption spectrum intensity of **ZS-1** can be observed by titration with more than 1 equivalent of Zn²⁺ per ligand. Upon the addition of Zn²⁺ to a **ZS-1** solution in MeCN, the quenching is disrupted by coordination of Zn²⁺ by the DPA moiety, and the **ZS-1** solution shows an intense green fluorescence [Figure 1(b)].



Scheme 1 Reagents and conditions: i, ClCH₂C(O)Cl, CH₂Cl₂, reflux, 1 h; ii, BF₃·Et₂O, Et₃N, PhMe; iii, di(2-picolyl)amine, KI, K₂CO₃, MeCN, reflux, 6 h.

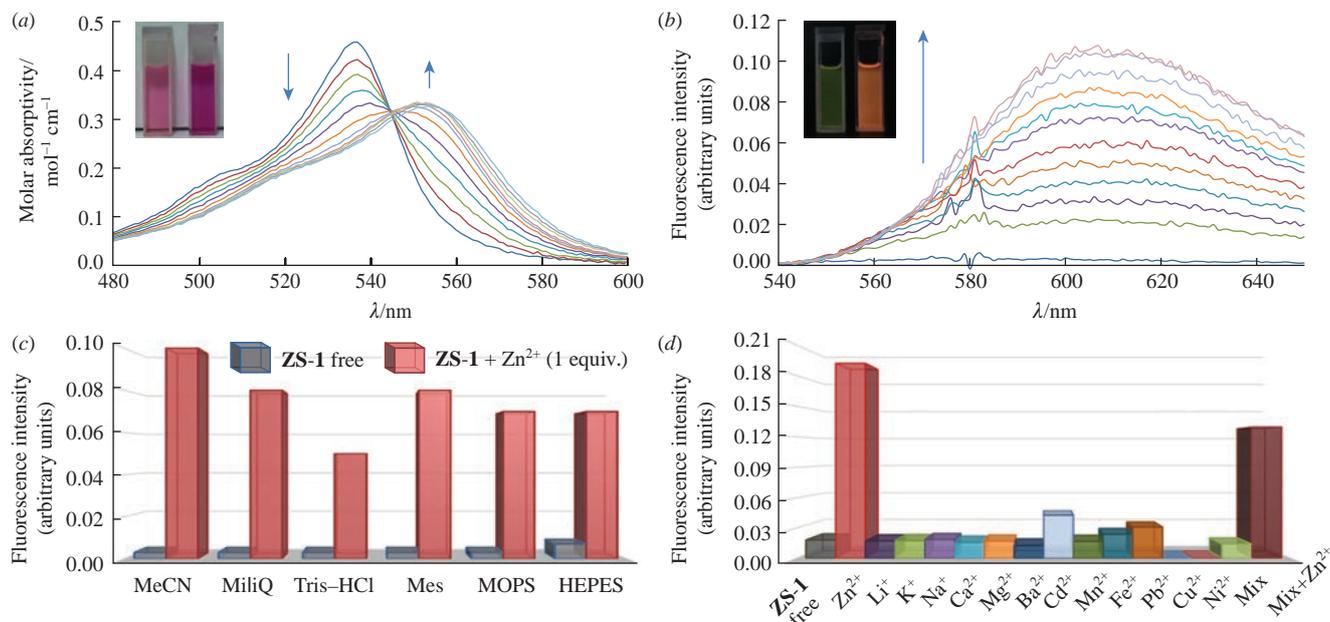


Figure 1 Spectral properties of **ZS-1** in the presence of metal cations: (a) absorption titration spectra of **ZS-1** upon the addition of Zn^{2+} (0–1.1 equiv.) in a 10^{-5} M MeCN solution; (b) fluorescence titration spectra of **ZS-1** upon the addition of Zn^{2+} (0–1.1 equiv.) in a 10^{-5} M MeCN solution ($\lambda_{\text{ex}} = 580$ nm); (c) fluorescent intensities of **ZS-1** (10 μM) upon the addition of Zn^{2+} (1 equiv.) in MeCN and 1 : 1 v/v mixtures of MeCN with miliQ water and the following buffer solutions: Tris–HCl (pH 7.5), MES (pH 6.0), MOPS (pH 7.5), HEPES (pH 7.5); (d) fluorescence intensities of **ZS-1** (10 μM) upon the addition of various metal ions (1 equiv.) in MeCN/ H_2O (1 : 3, v/v). Mix is mixture of **ZS-1** (10 μM) and cations (10 equiv.): Li^+ , K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Cd^{2+} , Mn^{2+} , Fe^{3+} , Pb^{2+} ; Mix+ Zn^{2+} is mixture of Mix and cation Zn^{2+} (1 equiv.).

The emission maximum of the **ZS-1** complex undergoes a strong red shift to 610 nm (excitation at 580 nm) and the quantum yield increases up to 21 times. The maximum fluorescence emission wavelengths (548 nm) are not shifted upon the addition of Zn^{2+} ($\lambda_{\text{ex}} = 520$ –550 nm), while strong fluorescence enhancement is observed by the naked eye.

The **ZS-1** fluorescence enhancement upon complexation with Zn^{2+} was screened in different buffer solutions [Figure 1(c)]. A strong increase in the quantum yield was observed in water, MES, MOPS and HEPES (~8–16 fold); in a Tris–HCl solution, the fluorescence enhancement decreases almost twice.

The fluorescence response of **ZS-1** to various cations and its selectivity for Zn^{2+} have been examined [Figure 1(d)]. Cations such as Li^+ , K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} and Mn^{2+} have no influence on the fluorescence enhancement. Among the transition metal cations, Fe^{2+} , Pb^{2+} and Cd^{2+} show only slight effect on the fluorescent enhancement upon the subsequent addition of Zn^{2+} . Metal ions with open-shell d-orbitals such as Ni^{2+} and Cu^{2+} act as quenchers of fluorescence of **ZS-1** via electron or energy transfer between the metal ion and the fluorophore.²³ To further explore the utility of **ZS-1** as an ion-selective fluorescent chemosensor for Zn^{2+} , competitive experiments were performed in the presence of Zn^{2+} and a mixture of Li^+ , K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Ba^{2+} , Cd^{2+} , Mn^{2+} , Fe^{2+} and Pb^{2+} . No significant changes in the fluorescence intensity were observed compared with that measured in the absence of other metal ions apart from Zn^{2+} . The above results are indicative of remarkable selectivity of **ZS-1** for Zn^{2+} ions.

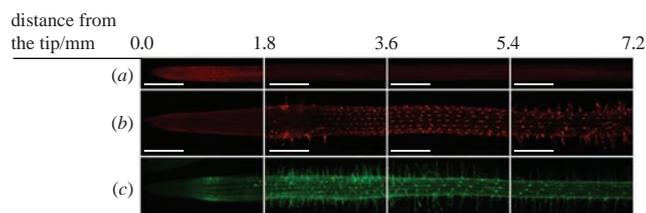


Figure 2 Bioimaging of *Brassica napus* L. roots treated with (a) **ZS-1** (control), (b) **ZS-1** and Zn^{2+} , (c) Newport GreenTM DCF sensor and Zn^{2+} . Scale bars represent 500 μm .

The **ZS-1** sensor was used to detect Zn^{2+} in root cells of the *Brassica napus* L. plants [Figure 2(a),(b)]. Under normal conditions, the sensor showed rather weak diffuse staining of root cells. On the contrary, the staining of plants grown in the presence of excess zinc (250 μM) for 3 days resulted in significantly brighter staining, with clearly visible brightly stained clusters in the cytoplasm.

The ability of the **ZS-1** sensor to specifically detect zinc ions *in vivo* was tested on roots of the *Brassica napus* plants grown under conditions of zinc, cadmium, cobalt, copper, nickel, lead or aluminum excess (see Online Supplementary Materials). Only zinc excess induced a characteristic increase in the fluorescence in canola roots mainly localized to root rhizodermis cells. Other metals did not cause a substantial rise in the fluorescence level compared to the control roots, and copper even caused a drastic decrease in the fluorescence level. Thus, **ZS-1** showed the ability to detect zinc excess in plant roots with high specificity.

The ability of **ZS-1** to measure intracellular labile zinc level was compared with that of the commercially available zinc sensor Newport GreenTM DCF [Figure 2(c)]. Both sensors exhibited similar patterns of root cells staining. The fluorescence of both sensors was considerably increased in hair rhizodermal cells localized at the periphery of the cells. However, **ZS-1** has a number of significant advantages over Newport Green DCF. First, Newport Green DCF has a quite high quantum yield in the metal-free form and displays only 3.3-fold increase in fluorescence upon zinc binding;²⁴ in contrast, **ZS-1** showed a 16-fold increase in the fluorescence intensity upon zinc binding. Therefore, the background tissue staining was quite low for **ZS-1**, being significant for Newport GreenTM DCF. The **ZS-1** sensor displays higher photostability than Newport GreenTM DCF and negligible leakage from the root cells. The **ZS-1** sensor has additional advantages due to excitation and emission in the orange-red spectral region, since long-wavelength radiation better penetrates plant tissues and has a less damaging effect. In addition, plant tissues have virtually no autofluorescence in this spectral region.

In conclusion, we described the synthesis, properties and *in vivo* application of **ZS-1**, a novel BODIPY-based fluorescent

chemosensor for Zn²⁺ imaging in living biological samples. Compound **ZS-1** is a highly efficient Zn²⁺-selective fluorescent probe with visible excitation and emission profiles. Spectral studies show that **ZS-1** exhibits properties similar to those of previously reported zinc probe,²² with the additional advantages like strongly red-shifted emission maximum, efficient naked-eye and UV-VIS detections. Imaging experiments for the *Brassica napus* L. roots also demonstrated the value of **ZS-1** in practical applications of the Zn²⁺ monitoring of biological systems. The described fluorescent probe can serve as a useful tool for localizing, visualizing, and studying the toxic effects of zinc in plants tissues.

This work was supported by the Russian Foundation for Basic Research (grant no. 17-53-16012) and the Moscow City Government in the framework of the research (project no. 15-34-70030 ‘mol_a_mos’).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2018.11.017.

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Received: 20th April 2018; Com. 18/5550