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A rhodamine-based chemosensor with diphenylselenium for highly selective fluorescent turn-on detection of Hg^{2+} in *vitro* and *vivo*

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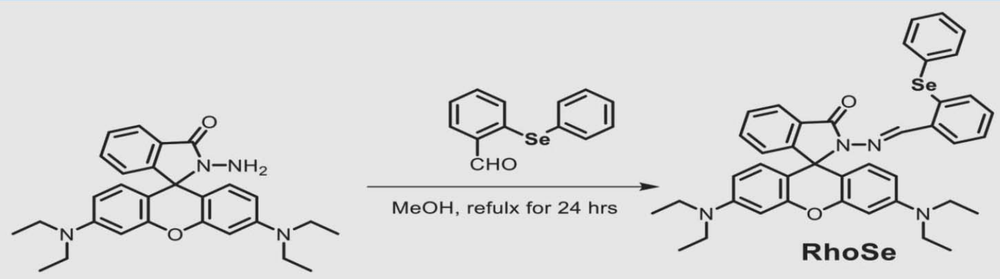
Abstarct

A rhodamine-B based chemosensor with diphenylselenium **RhoSe** has been synthesized, and its detecting behaviors towards various metal ions are studied via UV/Vis and fluorescence spectra. **RhoSe** shows colorimetric and fluorescent turn-on responses towards Hg^{2+}. The binding ratio of **RhoSe**-Hg^{2+} was determined by Job plots as a 1:1 ratio, and the effective pH range for Hg^{2+} detection was 4.0–10. Importantly, the reversibility of the **RhoSe**-Hg^{2+} complex was observed through the addition of Na_2S. For practical applications, the strip method was utilized to detect Hg^{2+} in water. In addition, cell imaging experiments demonstrated that **RhoSe** is an effective fluorescent probe for Hg^{2+} detection *in vitro* and *in vivo*.

Keywords: Hg^{2+} ions, Rhodamine, Test trip method, Bioimaging, Zebrafish

Synthesis route of RhoSe



Scheme 1.Synthesis route of **RhoSe**

Color change and Fluorescent response of RhoSe with Hg^{2+}

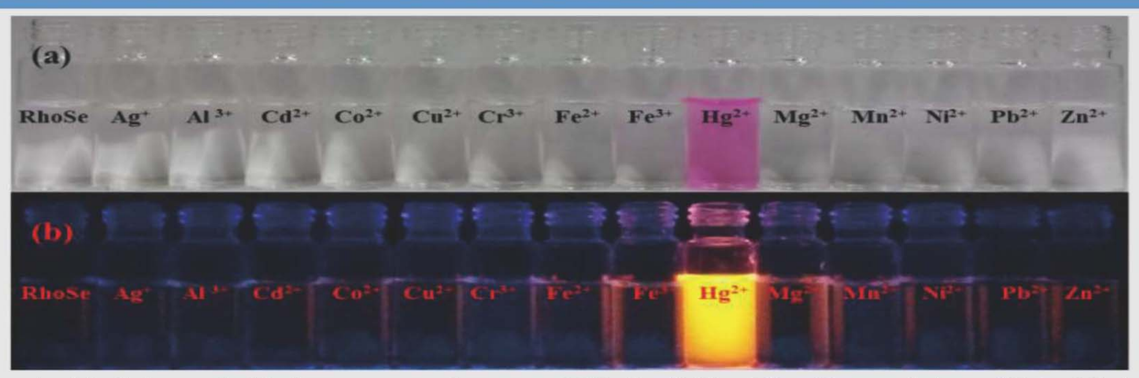


Fig. 1. (a) Color and (b) fluorescence changes of **RhoSe** (100 μM) with metal ions (100 μM) in CH_3OH/H_2O (v/v = 9:1) solution.

Fluorescence spectroscopic Selectivity test for RhoSe with Hg^{2+}

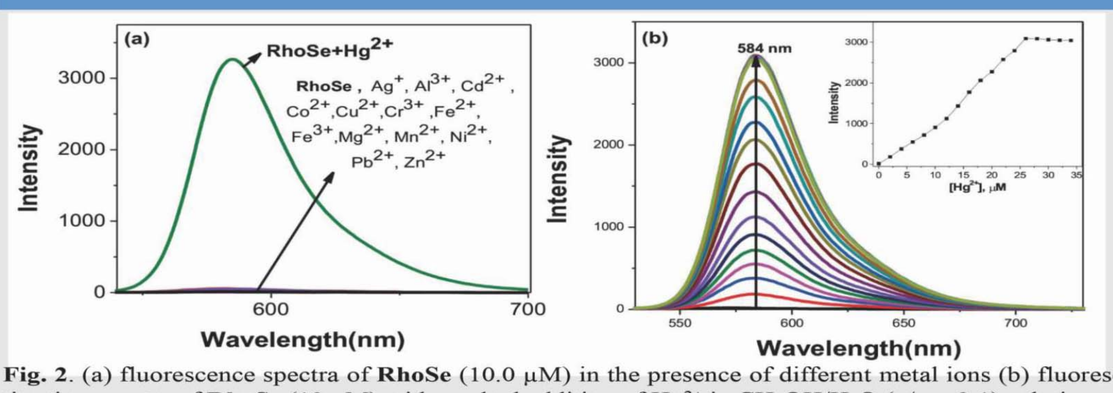


Fig. 2. (a) fluorescence spectra of **RhoSe** (10.0 μM) in the presence of different metal ions (b) fluorescence titration spectra of **RhoSe** (10 μM) with gradual addition of Hg^{2+} in CH_3OH/H_2O (v/v = 9:1) solution.

Competition test and Effect of pH

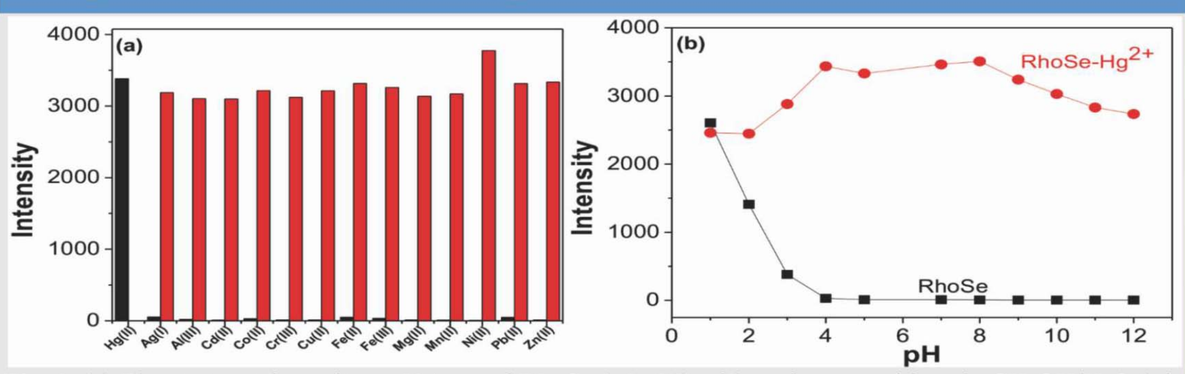


Fig. 3. (a) Fluorescence intensity at 584 nm of **RhoSe** (10 μM) with various metal ions in CH_3OH/H_2O (v/v = 9:1) solution. The black bars represent single metal ion (30 μM); the red bars represent coexisting metal ion:Hg^{2+} (30 μM) + other metals (60 μM). (b) Fluorescence intensity at 584 nm of **RhoSe** (10 μM) with Hg^{2+} (30 μM) at different pH in CH_3OH/H_2O (v/v = 9:1) solutions. The excitation wavelength was 510 nm.

Reversibility Test and Test trip method

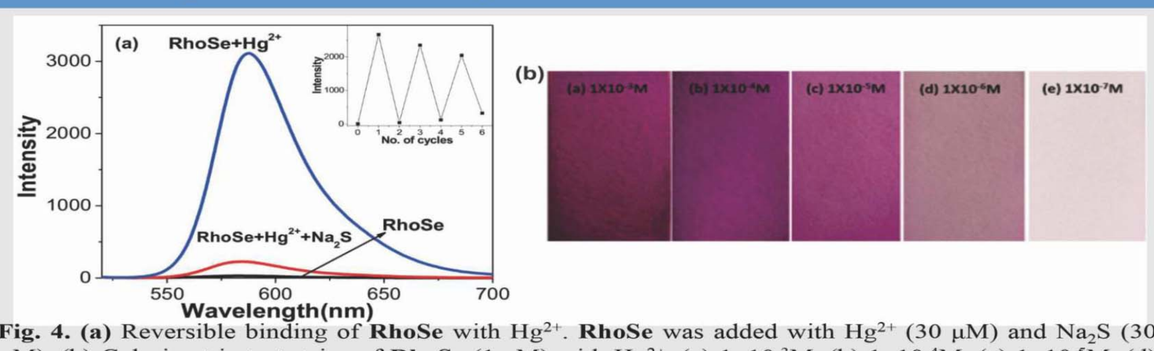


Fig. 4. (a) Reversible binding of **RhoSe** with Hg^{2+}. **RhoSe** was added with Hg^{2+} (30 μM) and Na_2S (30 μM). (b) Colorimetric test strips of **RhoSe** (1mM) with Hg^{2+}. (a) 1×10^{-3}M, (b) 1×10^{-4}M, (c) 1×10^{-5}M, (d) 1×10^{-6}M, (e) 1×10^{-7}M.

Bio imaging of RhoSe

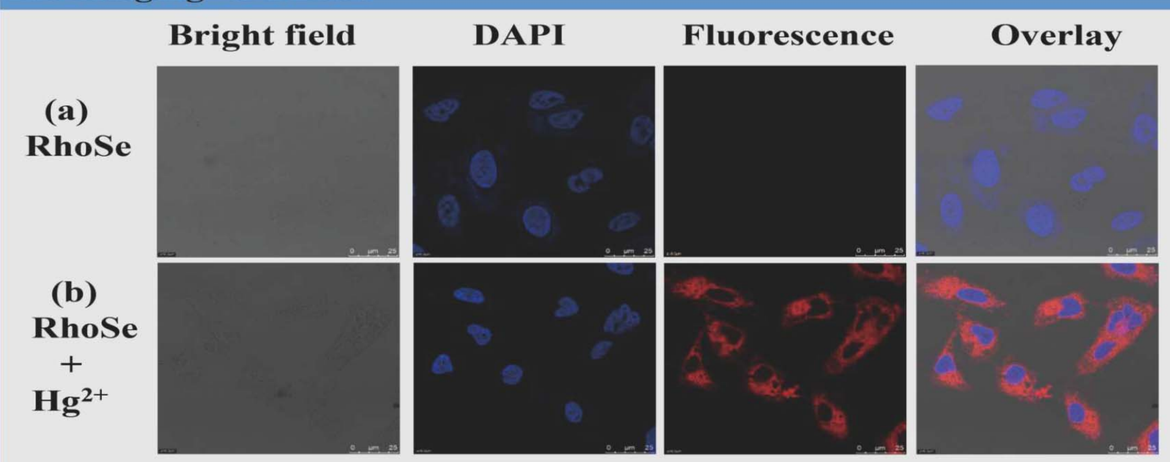


Fig. 5. Confocal microscopy images of **RhoSe** treated with Hg^{2+} ions in HeLa cells.

Confocal microscopy images of 3-day-old zebrafish

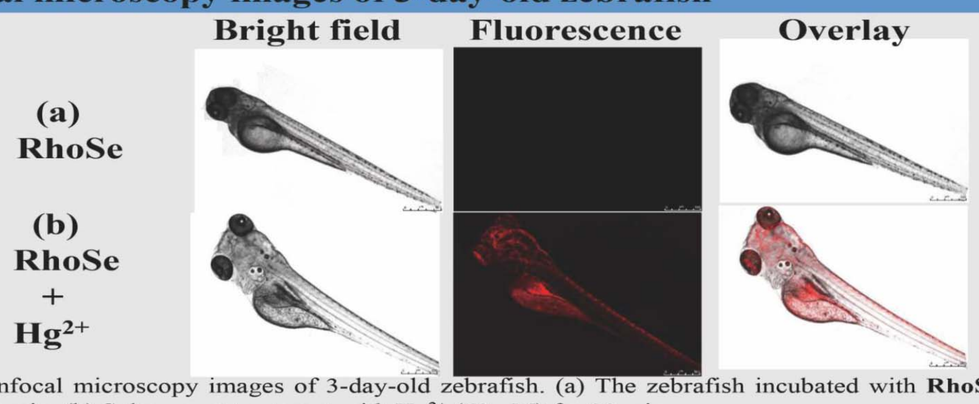


Fig. 6. Confocal microscopy images of 3-day-old zebrafish. (a) The zebrafish incubated with **RhoSe** (20 μM) for 30 min. (b) Subsequent treatment with Hg^{2+} (60 μM) for 30 min.

Confocal microscopy images of 3-day-old zebrafish Brain

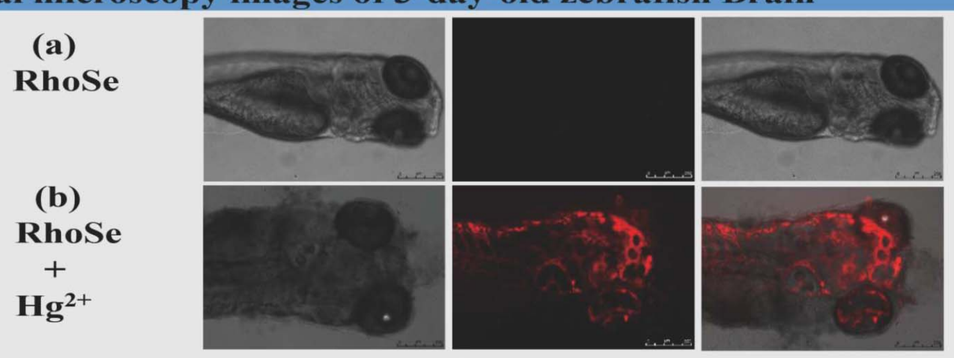


Fig. 7. Hg^{2+} accumulation and distribution in 3-day-old zebrafish brain.

Conclusion

In summary, a rhodamine-based probe **RhoSe** with diphenylselenium has been designed for selective and sensitive Hg^{2+} detection. **RhoSe** showed a rapid response to Hg^{2+} alone among other metal ions, with colorimetric and fluorescent turn-on responses. **RhoSe** can be used for Hg^{2+} detection over a pH range of 4.0–10.0. In addition, the stoichiometry of the **RhoSe**-Hg^{2+} complex was calculated as a 1:1 binding ratio using a Job plot, and was further confirmed by ESI-MS. **RhoSe** shows high sensitivity towards Hg^{2+} with a detection limit of 12 nM. Importantly, the practical application of **RhoSe** for Hg^{2+} detection was successfully demonstrated through test strips, live cell images, and live zebrafish images. **RhoSe** has been developed as a useful tool for monitoring Hg^{2+} distribution in biological samples.