

Supporting Information

Phenazine-imidazole based ratiometric fluorescent probe for Cd²⁺ ion and its application in vivo imaging

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Bio-imaging procedure

Live-cell fluorescence imaging: *Oxya chinensis* cells were grown at 37°C in Dulbecco's Modified Eagle Medium media (DMEM) supplemented with 10 % fetal bovine serum (FBS) under an atmosphere of 5% CO₂ and 95% air at 37 °C. For the imaging study, the cells were incubated first with 10 μM PIS for 20 min. After removal of excess PIS with PBS buffer, the cells were further treated with 20 μM Cd²⁺ for another 10 min, respectively. Again, the cells were rinsed with PBS buffer to remove unbound Cd²⁺ ions. After that the fluorescence images were collected using a 63×objective (excited at 405nm).

Zebrafish fluorescence imaging: Zebrafish (provided by School of Life Sciences, ShanXi University, China) were maintained in the facility according to the standard operational guidelines. Three-day-old larval zebrafish was pre-treated with probe PIS (10 μM) for 20min and then with 30μM Cd²⁺ for another 10 min. After rinsing the zebrafish with PBS buffer to remove remaining PIS and unbound Cd²⁺ ions, the fluorescence imaging was performed using a 5×objective (excited at 405nm).

imaging of growing *Arabidopsis thaliana* root: *Arabidopsis thaliana* plants were grown on Murashige–Skoog (MS) medium (containing vitamins and 1% sucrose) at 22°C under 16 h light/8 h dark conditions. All imaging experiments were performed using 5-day-old seedlings of *Arabidopsis thaliana* with a 63×objective (excited at 405nm). Before the imaging experiments, the whole seedlings were incubated first with 10 μM PIS for 15 min and then with Cd²⁺ (30μM) for another 15 min.

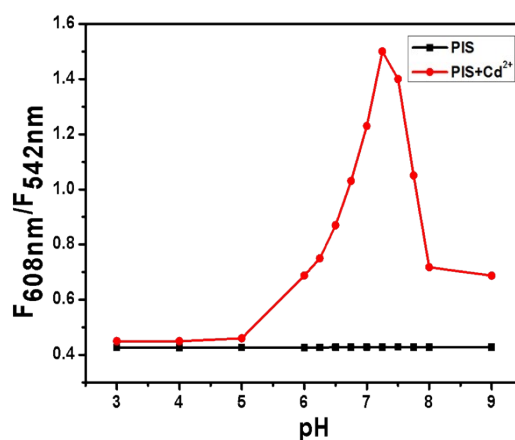


Fig.S1. pH-dependent fluorescence responses of PIS to Cd²⁺ (7 equiv.) in CH₃CN/H₂O (4:1) solution.

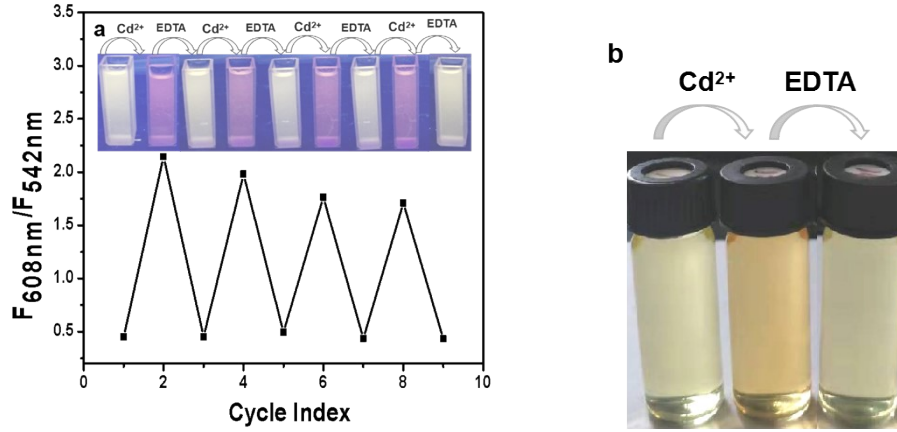


Fig.S2. Emission intensity changes and the accompanying color changes of PIS upon alternate addition of Cd^{2+} and EDTA. $[\text{Cd}^{2+}] = 28 \mu\text{M}$, $[\text{EDTA}] = 28 \mu\text{M}$.

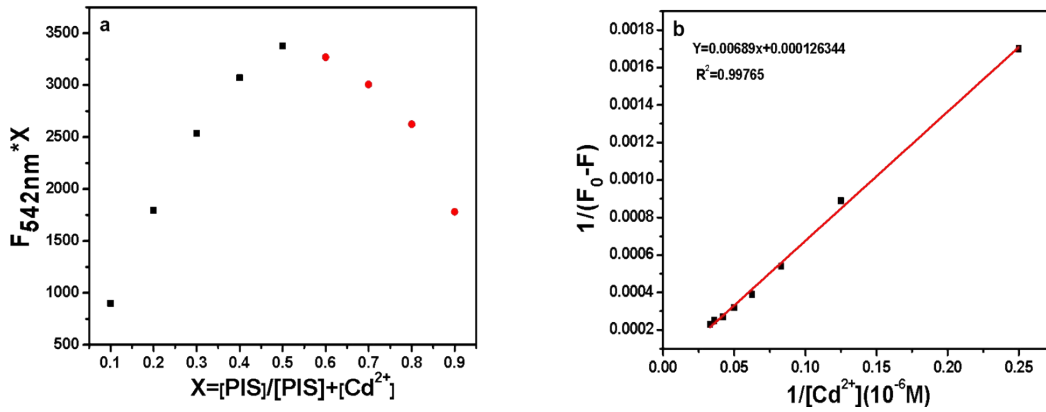


Fig.S3. (a) Job's plot for PIS and Cd^{2+} complexation in $\text{CH}_3\text{CN}/\text{HEPES}$ buffer (pH 7.4, v/v, 4:1). The total concentration of PIS and Cd^{2+} is $20 \mu\text{M}$. (b) Benesi-Hildebrand plot.

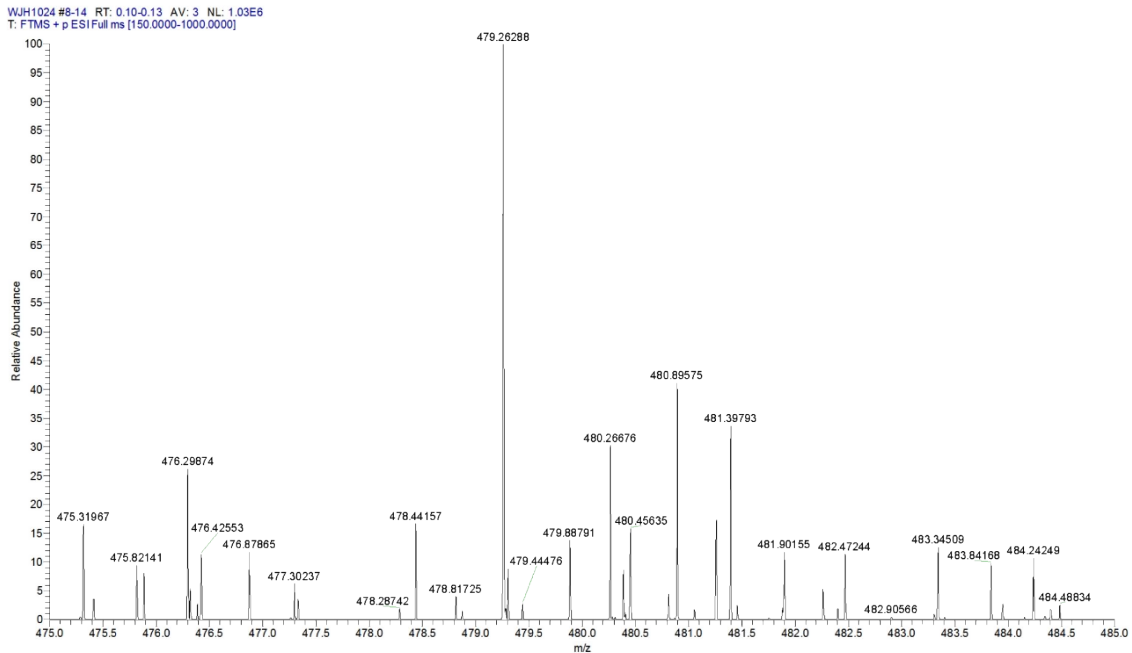


Fig.S4. ESI-HRMS of PIS upon addition of Cd^{2+} in CH_3CN .

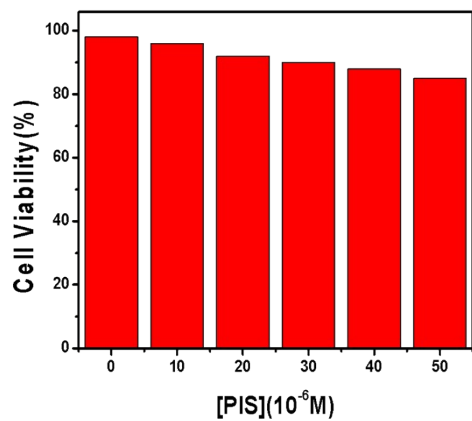


Fig.S5. Cell cytotoxic effect of PIS on SMMC-7721 cells.

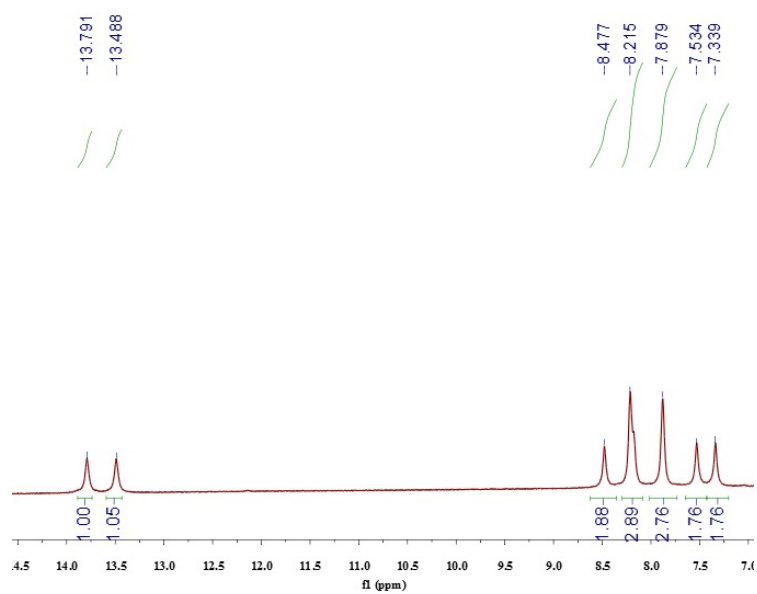


Fig.S6. ^1H NMR spectrum of sensor PIS

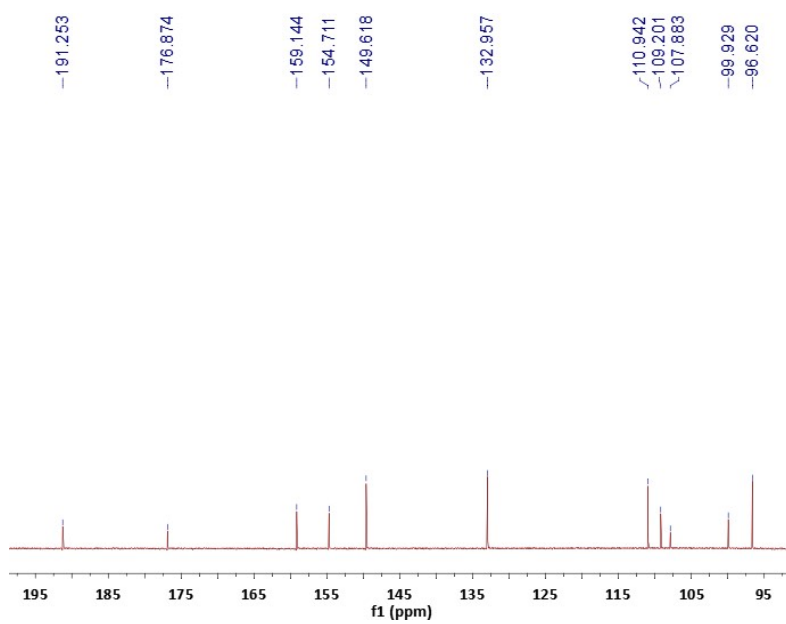


Fig.S7. ^{13}C NMR spectrum of sensor PIS

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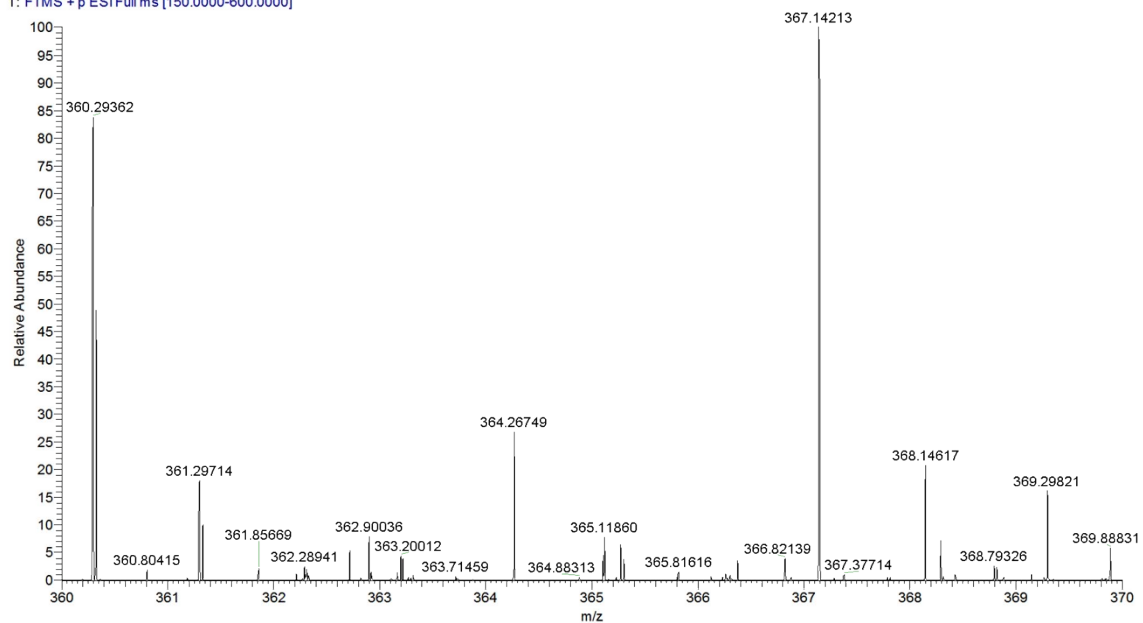


Fig.S8. Mass spectrum (ESI) of sensor PIS