An ICT-based fluorescence enhancement probe for detection of Sn$^{2+}$ in cancer cells†

Xiangying Meng,a Lai You,a Siyuan Li,a Qi Sun, b Xiaogang Luo,bc Haifeng He, id a Jinglan Wang* and Feng Zhao* a

Development of a novel fluorescence enhancement probe for detection of Sn$^{2+}$ in organisms, with high selectivity and sensitivity, is of great interest but remains a great challenge. Herein, an ICT-based fluorescence probe TPPB was rationally developed to act as an ‘enhancement’ luminescent and “naked-eye” indicator for Sn$^{2+}$ detection. Importantly, spectroscopic studies indicated that TPPB was a fluorescence enhancement sensor for Sn$^{2+}$ with rapid response, low detection limit (0.116 μM) and excellent binding constant (1.6 × 10$^4$ M$^{-1}$). The mechanism of TPPB response to Sn$^{2+}$ was further proved by $^1$H NMR titration, and enhancement calculations. Furthermore, TPPB is applied as a fluorescence probe for imaging in Hela cells, indicated that it can be potentially applied for Sn$^{2+}$ sensing in biological fields.

Sn$^{2+}$ ions have routinely been determined by flame atomic absorption spectrometry, 13 potentiometry, 14 voltammetry, 15 and UV-vis spectrophotometry. 16 Compare with these analytical approaches, fluorescent sensors have been developed for the determination of various important chemical species, as they offer significant advantages of excellent sensitivity, simplicity, instantaneous response and low detection limit up to nanomolar scale by contrast with routine analysis. 17–19 Therefore, the study of fluorescence probe as the high selectivity and sensitivity detection of Sn$^{2+}$ is urgently desirable. Hitherto many of fluorescence probes for the detection of Sn$^{2+}$, which sensing mechanisms are mainly based on chemical reaction, complexation reaction, quantum dots, and nanoparticles, have been established. 20–22 However, Sn$^{2+}$-selective sensors based on chemical reaction and complexation reaction have rarely been reported. 23 Fluorescence probes, which based on complexation reaction between the probe and analyte, have become more and more precise, sensitive and popular in research. While the fluorescence quenching probe is more common in research when metal ions are bound to fluorophore receptor system, but other interference factors could cause the quenching of the fluorescence so that its sensitivity and selectivity will be reduced and inferior to the fluorescence enhancement probe. 24,25 Therefore, the fluorescence enhancement probe is highly preferable than the fluorescence quenching probe for practical applications about detecting heavy metal ions like Sn$^{2+}$, due to the higher sensitivity, selectivity, and reliability. The fluorescence enhancement probe is also quenched in heavy metal ions detection via enhancement spin–orbit coupling energy or electron transfer. For overcoming this challenge, sensors structures are often designed to contain specific moieties such as triphenylamine. 26 Recently, we have reported a probe of 2-
imidazole-pyridine derivatives with emission at 446 nm by utilizing the nitrogen of imidazole and pyridine to form complex with metal ion, but it shown fluorescence quenched behavior after coordinating with metal ion (Fig. 1). In this work, Schiff base structural unit was designed to replace the imidazole to form weak electron-withdrawing center. Furthermore, diethylamino and triphenylamine moiety are strong electron-donating groups and thus are suitable as donor in an intramolecular charge transfer (ICT) system. The ICT process of electron-donating groups and thus are suitable as donor in an ICT system (API2000; Applied Biosystems, Foster City, CA, USA), recorded in parts per million (ppm) with TMS as the internal standard. The selectivity of the probe TPPB was prepared according to Scheme 1. Initially the intermediates M-1 was synthesized by the straightforward cross-coupling reaction under standard Suzuki coupling condition. The Schiff base sensor TPPB was prepared in 78.6% yield as yellow solid from the condensation of 5-(4-(diphenylamino)phenyl)picolin-aldehyde with N,N-diethylbenzene-1,4-diamine with metal ion salts of K+, Ag+, Cu2+, Pb2+, Sn2+, Mg2+, Mn2+, Hg2+, Cd2+, Ca2+, Ba2+, Cr3+, Al3+, Fe3+ (4.5 equiv.) in THF solution, respectively (Fig. 2A). Remarkably, only Sn2+ elicited a large fluorescence enhancement at 561 nm with possible interferences including metal ion salts of K+, Ag+, Cu2+, Pb2+, Sn2+, Mg2+, Mn2+, Hg2+, Cd2+, Ca2+, Ba2+, Cr3+, Al3+, Fe3+ (4.5 equiv.) (Fig. S1). 

Results and discussion
Synthesis and characterization
The sensor TPPB was prepared according to Scheme 1. Initially the intermediates M-1 was synthesized by the straightforward cross-coupling reaction under standard Suzuki coupling condition. The Schiff base sensor TPPB was prepared in 78.6% yield as yellow solid from the condensation of 5-(4-(diphenylamino)phenyl)picolinaldehyde with N,N-diethylbenzene-1,4-diamine in ethyl alcohol under refluxing condition. 

Optical analysis of probe TPPB sensing Sn2+
The selectivity of the TPPB probe for Sn2+ ion was researched by optical studies of the probe with various metal ions, and the sensitivity of the probe TPPB was also monitored by titration method. 

Experimental section
Materials and methods
All reagents for synthesis were analytically pure. All the solvents for spectroscopic measurement were chromatographically pure. 1H NMR spectra were recorded at 400 MHz, in CDCl3 solution and chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. Mass spectra (MS) were obtained on a QTRAP LC/MS/MS system (API2000; Applied Biosystems, Foster City, CA, USA), and signals were given in m/z. Fluorescence spectra were determined with a Hitachi F-4600 fluorescence spectrophotometer. Photoluminescence (PL) quantum yields were determined using a Hamamatsu system for absolute PL quantum yield measurements (type C11347).

Synthesis of compound TPPB
The synthetic method of compound TPPB was shown in Scheme 1. Compound 5-(4-(diphenylamino)phenyl)picolinaldehyde (M-1) was prepared according to the literature method. Ethanol (10 mL), N,N-diethylbenzene-1,4-diamine (0.173 g, 1.05 mmol) were stirred at room temperature, then, M-1 (0.35 g, 1.00 mmol) was added to the mixed solution under reflux for 2 h. After that the mixed solution was poured into 100 mL ice water, extracted with dichloromethane (3 × 30 mL), and evaporated under reduced pressure to remove the organic solvent. The product was isolated using silica gel column chromatography with petroleum ether/ethyl acetate = 5 as the solvent to yield faint yellow solid (0.39 g, 78.6% yield). 1H NMR ((400 MHz, CDCl3) δ (ppm): 8.89 (s, 1H, Py-H), 8.70 (s, 1H, CH= N), 8.21 (d, J = 8.3 Hz, 1H, Py-H), 7.94 (d, J = 8.3 Hz, 1H, Py-H), 7.52 (d, J = 8.2 Hz, 2H, Ar-H), 7.38 (d, J = 8.5 Hz, 2H, Ar-H), 7.29 (t, J = 7.6 Hz, 4H, Ar-H), 7.16 (t, J = 7.0 Hz, 6H, Ar-H), 7.07 (t, J = 7.3 Hz, 2H, Ar-H), 6.72 (d, J = 8.6 Hz, 2H, Ar-H), 3.41 (q, J = 7.0 Hz, 4H, CH3), 1.20 (t, J = 7.0 Hz, 6H, CH3); 13C NMR (100 MHz, CDCl3) δ (ppm): 154.57, 154.25, 148.70, 147.94, 147.82, 145.49, 139.15, 136.82, 134.37, 131.18, 129.79, 128.15, 125.56, 125.23, 123.84, 123.64, 121.64, 112.44, 44.96, 13.08; EI-MS m/z: [M + 1] 497.3. 

Fig. 1 Molecular structure of probe TPPB.
properties and inappropriate diameters of different metal ions. In Fig. 2C, the free TPPB can be seen as yellow solution. The color of the solution underwent change from yellow to colorless and transparent in the presence of Sn\(^{2+}\), however other metal ions did not bring about any obvious change in the color of the solution, except that Zn\(^{2+}\), Cd\(^{2+}\), and Hg\(^{2+}\) brought about with yellow enhancement. So, TPPB can be used as visual indictor for Sn\(^{2+}\). This technique is therefore superior to other analytical techniques because it has ability to detect Sn\(^{2+}\) by naked-eye.

Moreover, in order to explore the utility of TPPB as an ion-selective sensor, the competition experiments were carried out by adding other competitive metal ions to TPPB solution in presence of Sn\(^{2+}\) (Fig. 3). The black bar corresponds to the fluorescence probe TPPB with all of metal ions respectively, and red bar corresponds to the fluorescence probe TPPB with all of metal ions in presence of Sn\(^{2+}\) respectively. Interestingly, Sn\(^{2+}\) induced fluorescence responses were hardly influenced by these common coexistent metal ions. The evidence revealed that the probe TPPB shown a high selectivity and good stability toward to Sn\(^{2+}\) even in the presence of other relevant metal ions.

The sensitivity of the probe TPPB was monitored by titration analysis. The fluorescence titrations of TPPB with Sn\(^{2+}\) were

![Scheme 1](image1.png)

**Scheme 1** The synthetic pathways of probe TPPB.

![Fig. 2](image2.png)

**Fig. 2** (A) Fluorescence spectral of probe TPPB (20 μM) in the presence of Sn\(^{2+}\) (4.5 equiv.) and various of other metal ions (4.5 equiv.) in THF solution at room temperature, λ\(_{ex}\) = 420 nm; (B) the color changes of TPPB solution (20 μM) when a various metal ions (4.5 equiv.) were added under UV-light; (C) naked eyes color changes of the probe with different metal ions.
performed. As shown in Fig. 4A, probe TPPB exhibited extremely feeble fluorescence. However, with the addition of 0–4.5 equiv. of Sn$^{2+}$, the fluorescence intensity showed an excellent enhancement (up to 63-fold) at 561 nm. The fluorescence enhancement could be attributed to the strong ICT process.

The large fluorescence enhancement was corroborated by the observation that the fluorescence color of the sensor solution turned from very pale yellow to deep yellow (Fig. 4A, inset), which indicated that the probe TPPB showing an excellent fluorescence enhancement behavior for selective detection of Sn$^{2+}$ ion. Subsequently, an evident increasing observed in the quantum yield ($\Phi$) of [TPPB + Sn$^{2+}$] (68.21%) as compared to that of TPPB (1.43%) by using fluorescein ($\Phi_{\text{ref}} = 90\%$) as a standard fluorescence reference (the calculation formula see ESI S2.1†). It supports the fluorescence enhancement of TPPB observed in presence of Sn$^{2+}$, and suggesting a sensitive and selective detection of Sn$^{2+}$ by TPPB compared to other metal ions. Importantly, the sensor shown a good linear relationship between the fluorescence intensity at 561 nm and the concentrations of Sn$^{2+}$ from 1 to 6 $\mu$M (Fig. 4B), suggesting that sensor TPPB is potentially useful for quantitative determination of Sn$^{2+}$ with a large dynamic range.

In order to confirm the binding stoichiometry between TPPB and Sn$^{2+}$, the Job’s plots analysis was carried out. The plot of fluorescence intensity against the molecular fraction of [TPPB]/[Sn$^{2+}$ + TPPB] was provided in Fig. S4A,† it showed the minimum at mole fraction of 0.5 indicating 1 : 1 stoichiometry between TPPB and Sn$^{2+}$ in the complexes. Based on a 1 : 1 binding mode, apparent association constant ($K_a$) of the TPPB-Sn$^{2+}$ interaction was calculated from the fluorescence titration spectra using the formula shown in calculation S2.3 (see ESI S2.3†) and $K_a$ value was found to be $1.6 \times 10^4$ M$^{-1}$. What’s more, a good linear relationship of the fluorescence intensity as a function of [Sn$^{2+}$] concentration from 0–4.5 equiv. ($R = 0.99367$) was obtained (Fig. S4B†). From the slope of the linear fit, the limit of detection (LOD) of the probe TPPB for Sn$^{2+}$ ion was determined by using the formula shown in calculation S2.4 (see ESI S2.4†) and was found to be 0.116 $\mu$M (Fig. S4C†). Shortly, the probe TPPB could be a sensitive fluorescence probe for the quantitative detection of Sn$^{2+}$ at micromole levels.

To further confirm the binding mechanism of Sn$^{2+}$ with sensor TPPB, 1H NMR titration experiments were performed in CDCl$_3$ and shown in Fig. 5. The changes in 1H NMR signals of TPPB are more apparent with the appearance of the new peak around 10.10 and 8.99 ppm when the analyte concentration is 0.2 equiv., and subsequently, it becomes gradually intensified with further additions. Owing to the precipitation, we were unable to continue the experiment beyond 1.5 equiv. of Sn$^{2+}$ ions. The new signals are assigned to the proton of H$_a$ and H$_c$, which are located in the vicinity of Sn$^{2+}$ binding site. The high field shift of 0.09 ppm for the arene proton (H$_b$) shows that the electron density has decreased around H$_b$ after Sn$^{2+}$ binding, due to the $\pi$-electron density shifted to the carbon, which link with N. On the other hand, the chemical shi$\text{f}$ of H$_a$ and H$_c$ shifted from 7.93(d) and 8.21(d) ppm to 8.01(s) ppm. These results indicated that N acted as electron donors for coordination to Sn$^{2+}$. The data further proved that the TPPB coordinating with Sn$^{2+}$ with a stoichiometric ratio of 1 : 1.

DFT calculations

DFT studies further support the Sn$^{2+}$-assisted ICT process in TPPB. The optimized geometries of TPPB and TPPB-Sn$^{2+}$ adduct, which were identified with the Job’s plots analysis and 1H NMR titration experiments, have been generated using B3LYP/6-311G(d, p) level (Lanl2dz for Sn) with Gaussian 09 software.41 As shown in Fig. 6,
the N1 and N2 were found to be arranged in an almost in-plane orientation in TPPB and TPPB-Sn\(^{2+}\) adduct, with a dihedral angle of \(-179.3^\circ\) and \(1.9^\circ\), respectively. The planar conformation provides efficient \(\pi\)-conjugation. After optimizing structure of TPPB, the N1 and N2 were trans-configuration, while they convert to cis-configuration in TPPB-Sn. The above phenomenon means that the ground-state geometry undergoes a significant twist upon the addition of Sn\(^{2+}\).

Examination of the frontier molecular orbitals given in Fig. 7 suggests that the electron density in the HOMO and LUMO for TPPB is entirely localized on the pyridine, Schiff base and one benzene ring of triphenylamine moiety. The above phenomenon clearly demonstrates an obstruction of the ICT process in TPPB, resulting in a weak fluorescence emission. While after Sn\(^{2+}\) ion binding with TPPB, the electron density localized on the Schiff base and triphenylamine moiety in the HOMO then shifted to Sn\(^{2+}\) binding site during transition to LUMO, which indicates an ICT from the Schiff base and triphenylamine moiety to the Sn\(^{2+}\) binding site, resulting in a strong fluorescence emission. Moreover, the calculated energy difference between HOMO and LUMO (0.53 eV) of TPPB-Sn\(^{2+}\) is lower than that of the free TPPB (the HOMO to LUMO energy gap is 3.18 eV). Hence, the ICT process of TPPB-Sn is also energetically favorable.
Imaging of cancer cell

In order to demonstrate the potential application of TPPB for the detection of Sn²⁺ in biological media, fluorescence microscopy studies were carried out by using Hela cells. The cytotoxicity of TPPB against Hela cells and normal cells was measured on MTT assay. The appropriate result was achieved based on Hela cells and normal cells, which showed high livability with more than 80% survival after 24 h (Fig. S5†). It means probe TPPB shows low cytotoxicity against cells.

Furthermore, TPPB as the fluorescence probe imaged in Hela cells for detection Sn²⁺ was studied. As shown in Fig. 8, incubation of Hela cells with 10 μM of the probe TPPB for 30 min gave very low dim fluorescence in the intracellular region (Fig. 8B). After treatment with 100 μM Sn²⁺ for 30 min, the fluorescence intensity obvious enhancement in Hela cells (Fig. 8E). These results inferred that the fluorescence realized enhancement due to the intracellular uptake of Sn²⁺ result in form of complex TPPB-Sn²⁺. The above results providing direct

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**Fig. 7** HOMO–LUMO energy diagrams of TPPB with Sn²⁺ ions.

**Fig. 8** Fluorescence imaging of Hela cells incubated with TPPB (20 μM) for 30 min (A–C), and then with Sn²⁺ (200 μM) for another 30 min (D–F), (Left) bright filed image, (Middle) yellow channel, (Right) merge image; (G) the cytotoxicity of TPPB against Hela cells incubated with different concentration of TPPB for 24 h.
1.6 detection limit of 0.116.

In conclusion, we have synthesized and characterized probe TPPB for detection Sn^{2+}. Interaction of Sn^{2+} with TPPB induces the fluorescence enhancement response with emission at 561 nm due to ICT process. Probe TPPB, which shows low detection limit of 0.116 μM and strong association constant of 1.6 × 10^4 M^{-1}, is a highly sensitive and selective sensor toward Sn^{2+}, as demonstrated by selective, competitive and titration experiment. It could form complexes with Sn^{2+} with a stoichiometric ratio of 1 : 1. Importantly, the color of the probe TPPB changed from yellow to colorless and transparent with Sn^{2+} added under visible light, which meant that the probe can be used as visual indicator for Sn^{2+} by naked-eye. The possible recognition pattern was derived from DFT calculations, and ^1H NMR titration. Impressively, the probe TPPB with low toxicity has practical application in cell imaging, which indicated that the probe is suitable for tracking intracellular Sn^{2+}. In conclusion, TPPB can be used as a great promise candidate sensor for detection of Sn^{2+} in complex living samples.

Conflicts of interest

There are no conflicts to declare.

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