Supporting Information of

An ICT-based fluorescence enhancement probe for detection of Sn²⁺ in cancer cells

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S1. General information

Unless stated otherwise, all analytical grad chemicals and solvents used in this paper were purchased from commercial vendors. The salts used in stock solutions of metal ions were Na_2SO_4, KNO_3, $AgNO_3$, $Pb(NO_3)_2$, $CoSO_4·7H_2O$, $ZnSO_4·7H_2O$, $MgSO_4$, $NiSO_4·6H_2O$, $CuSO_4·5H_2O$, $FeSO_4·7H_2O$, $MnSO_4·2H_2O$, $Cr(NO_3)_3·9H_2O$, $Al(NO_3)_3·9H_2O$, $Fe(NO_3)_3·9H_2O$ and $Sn(ClO_4)_2·3H_2O$. The ¹H and ¹³C NMR spectra were collected on a Bruker AV-400(400 MHz) in a DMSO-d_6 solution with TMS as the internal standard. Mass spectrometry was recorded with a Finnigan LCQ mass spectrometer and an Agient 1200 LC/MSD mass spectrometer, and signals were given in m/z. Elemental analysis (**EA**) was obtained on a Vario ELIII CHNSO elemental analyzer. UV–vis absorption spectra were recorded on a Perkin Elmer Lambda-900 spectrophotometer. Fluorescence spectra was determined by a Hitachi F-4600 fluorescence spectrophotometer. Photoluminescence (PL) quantum yields were carried out using a Hamamatsu system for absolute PL quantum yield measurements (type C11347).

S2. Calculations

S2.1 Determination of Quantum Yield

The fluorescence quantum yield was deduced by the following equation[1, 2],

$$\Phi_x = \Phi_s \times \frac{D_x}{D_s} \times \frac{A_s}{A_x} \times \frac{\eta_x^2}{\eta_s^2}$$

Here the relative quantum yield of the probe and probe metal complex was determined by using fluorescein as standard fluorescence mean, with quantum yield of $\Phi_{ref} = 0.90$ (in NaOH). Where the notations in the above equation such that, Φ is the fluorescence quantum yield, D is the area under the emission spectra at $\lambda_{em}= 446$ nm, A is the absorbance at the excitation wavelength $\lambda_{ex} = 350$ nm, x subscript denotes unknown compound, and s as standard reference and η is the refractive index of the solvents used.

S2.2 Determination of Stoichiometry by Continuous Variation Plot (Job's plot) Measurement

The stoichiometric binding ratio of probe **TPPB** and Sn^{2+} ion was confirmed by continuous variation emission analysis[3-5] at λ_{em} 675 nm, the resulting data was plotted as change in fluorescence intensity, emission intensity in the vertical axis against the mole fraction, $X_{Sn^{2+}}$ of Sn^{2+} ions in the horizontal axis.

S2.3 Determination of Binding Constant

The binding constant of the **TPPB + Sn^{2+}** complex formed in solution has been determined by using the standard Benesi-Hildebrand (B-H) equation[3-5].

$$\frac{1}{F - F_o} = \frac{1}{K_a(F_{max} - F_o)\,[Sn^{2+}]} + \frac{1}{F_{max} - F_o}$$

Where, F_o is the fluorescence intensity of free probe **TPPB**, F is the observed fluorescence intensity at any given concentration of Sn^{2+}, F_{max} is the intensity at saturation point with the Sn^{2+}, K_a is the association constant and $[Sn^{2+}]$ is the concentration of the Sn^{2+} ions in micromolar.

S2.4 Determination of Detection Limit

The detection limit was calculated based on fluorescence titration as a function the solubility of Sn^{2+} at λ_{em} 446 nm. The fluorescence emission spectrum of free probe **TPPB** was measured over 3 times to determine standard deviation for blank measurement. A linear plot was constructed with average values of the intensities against the concentration of Sn^{2+} ions for determining the slope. Using the slope the detection limit was calculated from the following equation[3, 5, 6].

$$LOD = \frac{3\sigma}{K}$$

where, σ is the standard deviation of the blank solution and K is the slope between intensity versus sample concentration.

S3. Cell Cultures and Imaging

Hela Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and penicillin-streptomycin (0.5U·mL^{-1} of penicillin and 0.5 g·mL^{-1} strepomycin) on a cell culture flask at 37℃ in an atmosphere of air with 5% CO_2 and constant humidity. Each cell line was seeded in a 6-well plate for 24 h. The cells were initially incubated with **TPPB** (20 μM) in culture medium for 30 min at 37℃. After washing three times with PBS to remove the remaining **TPPB**, the Hela cells were incubated in the absence and presence of Sn^{2+} (200 μM) in culture medium for another 30 min at 37 ℃. The imaging was carried out using inverted fluorescence microscopy (Olympus IX71, Japan).
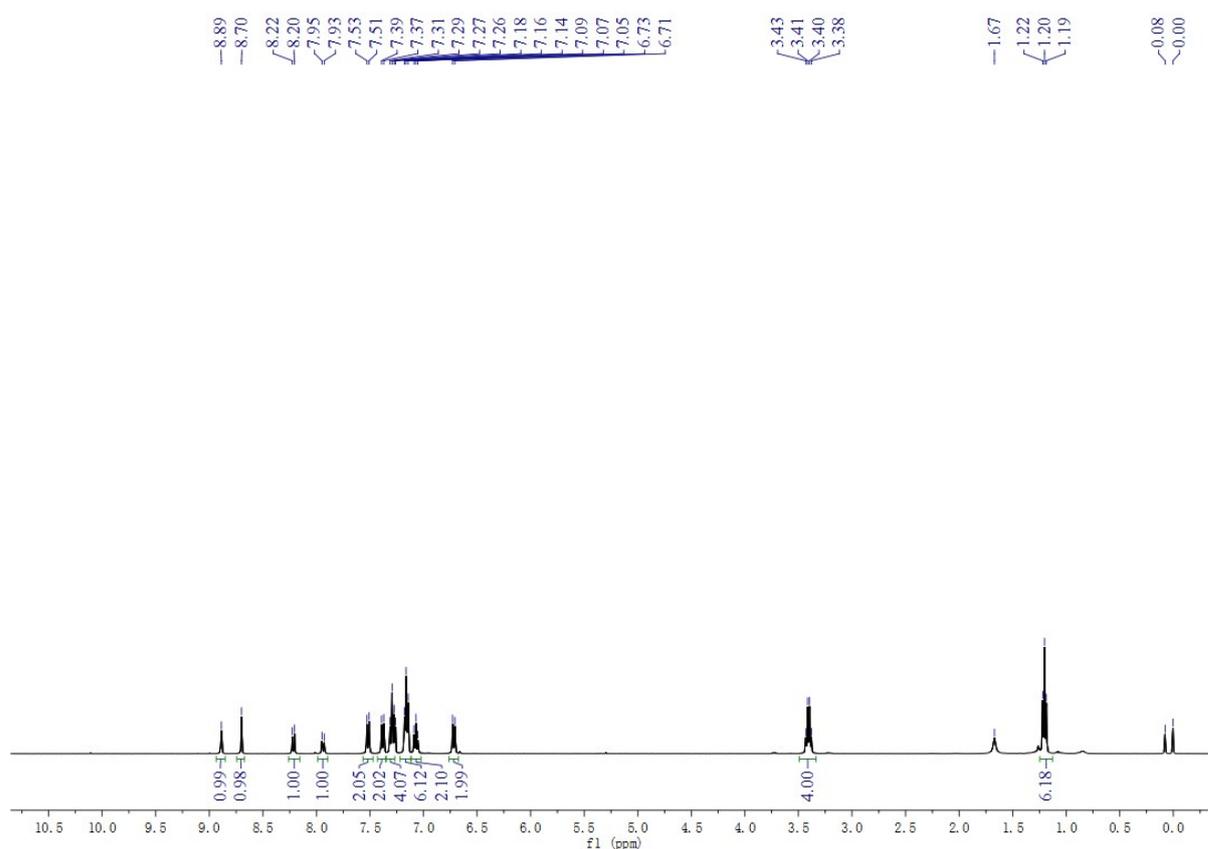
Fig. S1. ^{1}**H NMR** Spectrum of probe **TPPB** in CDCl$_3$

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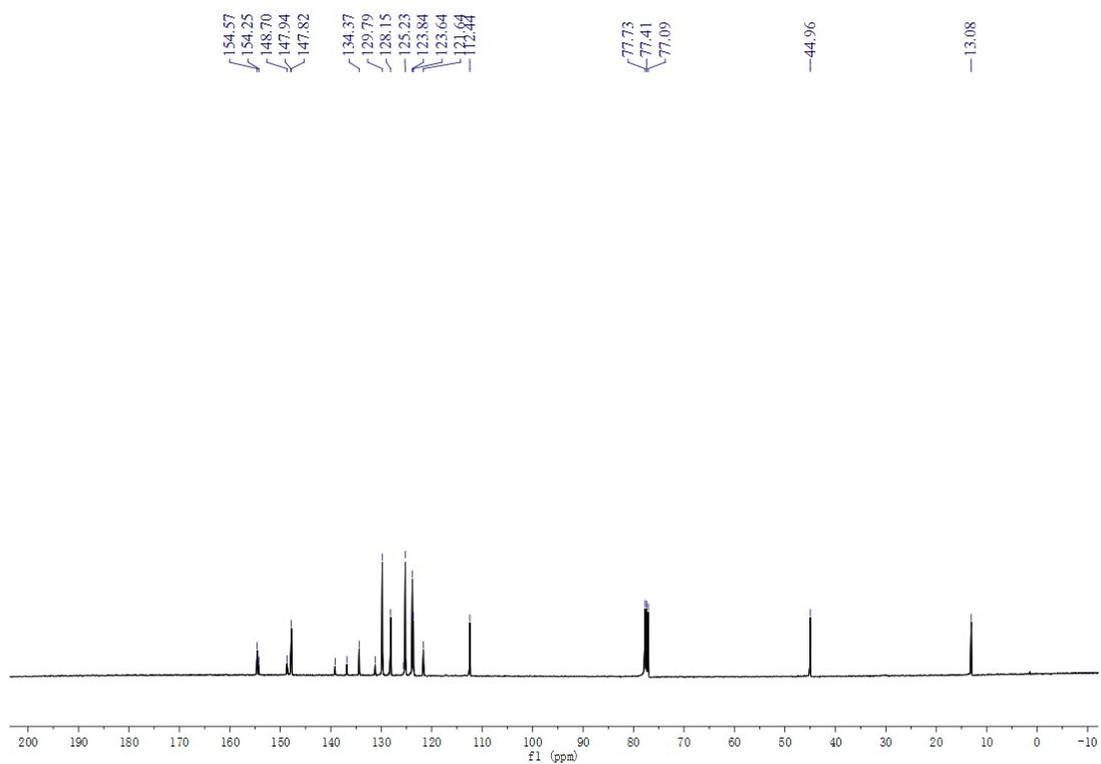
Fig. S2. ¹³C NMR Spectrum of probe **TPPB** in CDCl₃
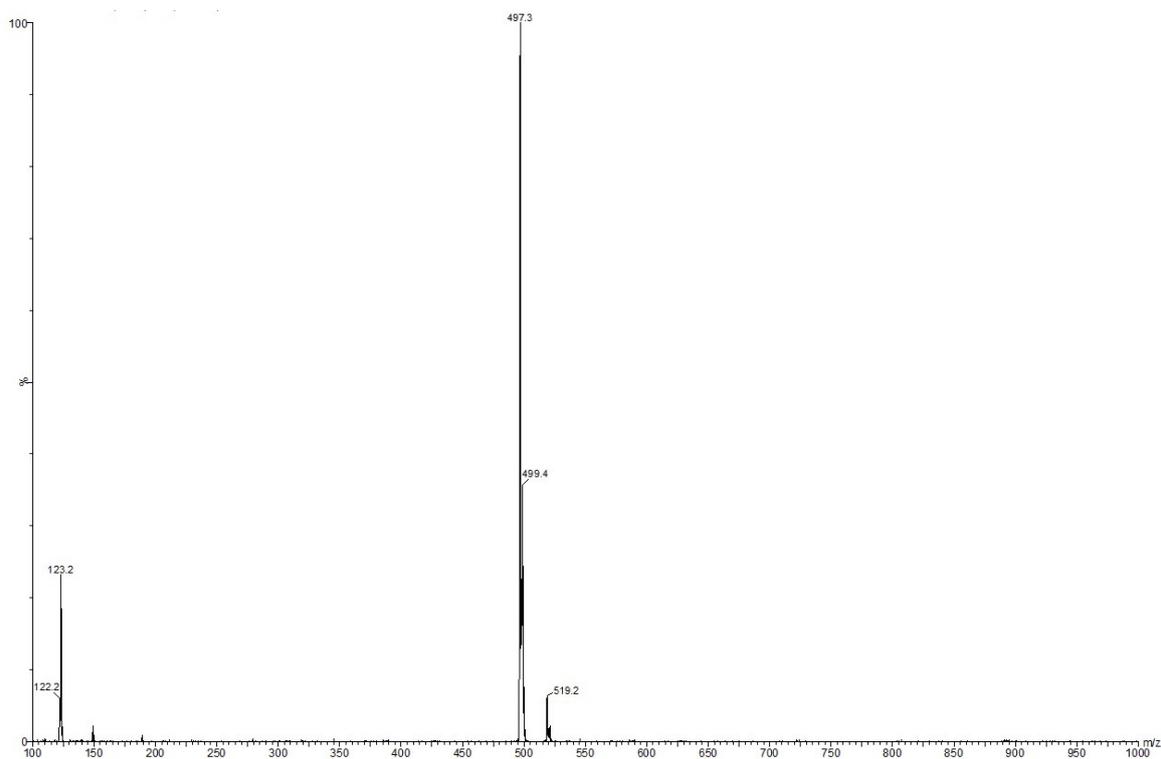
Fig. S3. MS Spectrum of probe **TPPB**
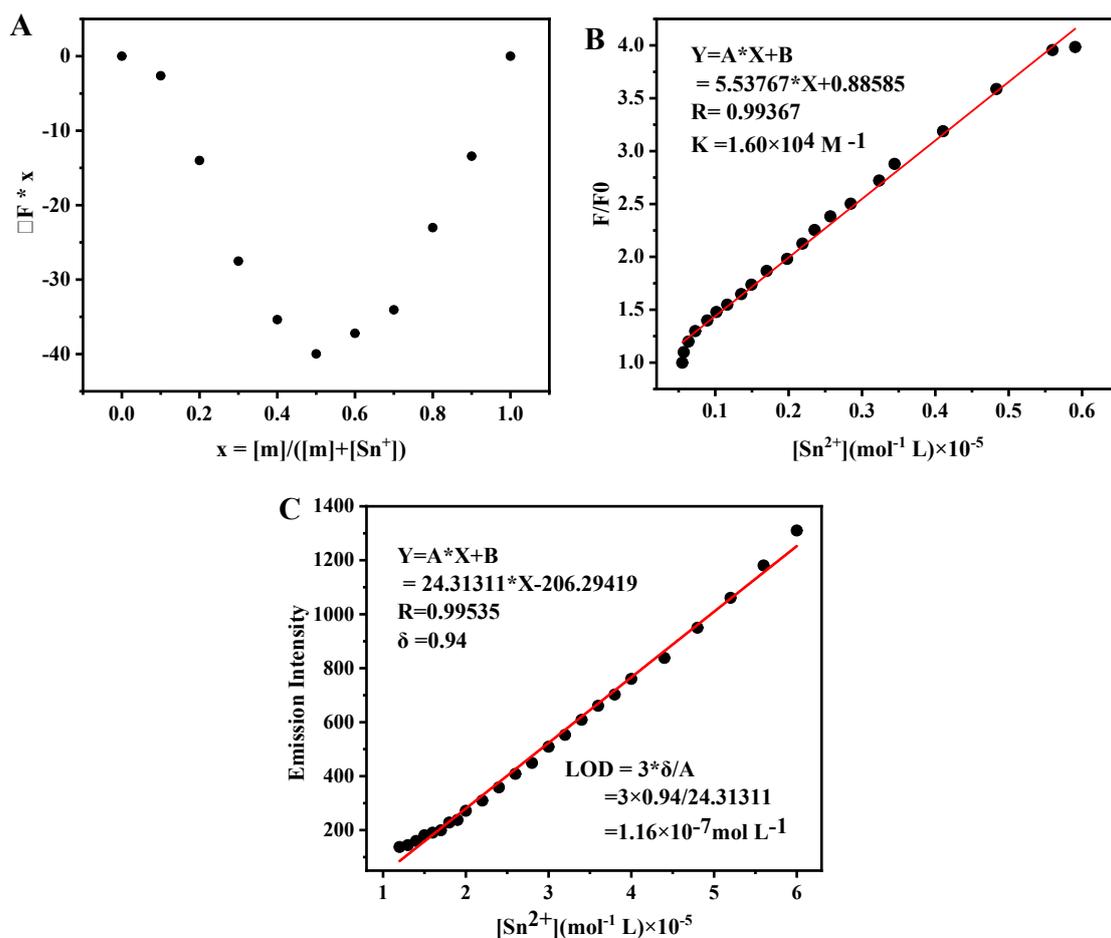
Fig. S4 (A) Job's plot for the complex of **TPPB** with Sn^{2+} in THF; (B) The association constant (Ka) is 1.6×10^4 M^{-1}; (C) The limit of detection (LOD) is 0.116 μM.
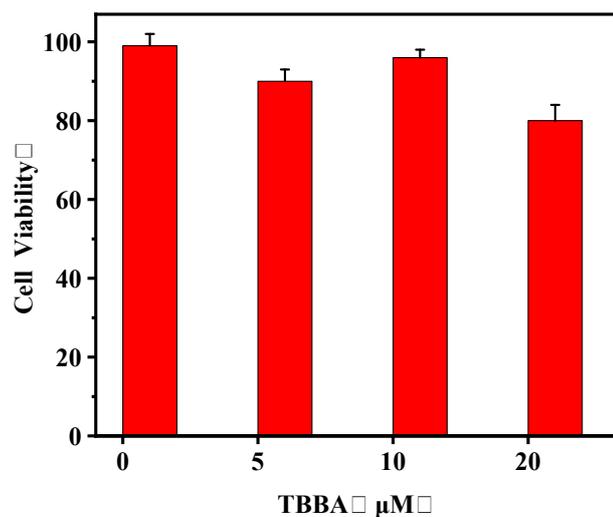
Fig. S5. The cytotoxicity of **TPPB** against normal cells incubated with different concentration of **TPPB** for 24 h

References

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