Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Triphenylamine schiff base as a lipid droplet-targeted fluorescent probe using Si-O-Si as bridge for the detection of Cr^{6+} applied in bio-imaging

Xiaoni Wang,Yujing Zuo,Yu Zhang, Tingxin Yang,and weiying,Lin*

Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Materials Science and Engineering, University of Jinan, Shandong 250022, P.R. China

Email: weiyinglin2013@163.com.

Table of contents

Page

1. Materials

All chemicals and solvents were of analytical grade and were used without further purification.4-(Diphenylamino) benzaldehyde was purchased from Shanghai Saan Chemical Technology.3,3'-(1,1,3,3-tetramethyldisiloxane-1,3-diyl) bis (Propan-1-amine) purchased from Hangzhou Da di Chemical . The HeLa cells line were purchased from Procell Life Science&Technology Co,.Ltd. The other reagents used in this work were purchased from the supplier, and the water used in the experiment was ultrapure water.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.20 (s, 1H), 7.60 (d, J = 8.7 Hz, 2H), 7.36 – 7.23 (d, J=7.0 Hz, 4H), 7.14 (d, J = 7.3 Hz, 4H), 7.09 (t, J = 7.6 Hz, 4H), 3.66 – 3.54 (q, 2H), 1.73–1.69 (m, J = 15.1, 7.3 Hz, 2H), 0.60 – 0.56 (m, 2H), 0.10 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 160.2, 149.9, 147.2, 130.0, 129.4, 129.1, 125.1, 123.6, 122.2, 65.0, 25.0, 16.1, 0.4

MS (EI) m/z calculated for $C_{48}H_{54}N_4OSi_2$: 759.39, Found: 759.3909.

1.1 Absorption and fluorescence measurements

All experiments were performed in aqueous solution (25 μM PBS buffer, pH = 7.4, containing 10% ethanol). The probe Si-LDS (100 mM) stock solution was prepared in ethanol and the probe was then diluted to 10 mmol standby. And 100 mmol cation ions (Cr^{6+}/ Al^{3+}/ Fe^{3+}/ Ag^{+}/ Co^{2+}/ Sn^{2+}/ Zn^{2+}/ Ca^{2+}/ Mg^{2+}/ Ba^{2+}/ Ni^{2+}/ Cr^{3+}/ Mn^{2+}/ Cd^{2+}/ Cu^{2+}) were prepared in aqueous solution. The fluorescence spectra were obtained by excitation at 365 nm. The excitation and emission slit widths were 5 nm and 5 nm respectively.

1.2 Cell incubation and fluorescence imaging

HeLa Cells were maintained in an incubator at 37 °C under 5% CO_2 environments. HeLa Cells were cultured in 35 mm glass-bottomed dishes at a density of 3 × 10^5 cells per dish in culture media. After culture for 24 h ,the wastes were removed and washed with phosphate buffered saline (PBS) buffer for 2 times. HeLa cells were treated with 10 μM probe **Si-LDS** for 30 min, Cell images were passed through a Nikon A1MP confocal microscope. Fluorescence emission was obtained at

510-550 excitation at 405 nm. To test co-localization with the LDs, HeLa cells were incubated with 10 μM of probe **Si-LDS** and Nile Red (0.5 μM) for 30 min and fluorescence image acquired by confocal microscopy.

2. Instruments

MTT was obtained from Sigma-Aldrich. The ^{1}H NMR and ^{13}C NMR spectra were measured on an AVANCE III 400 MHz Digital NMR Spectrometer, and using $CDCl_3$ as solvent. Fluorescence spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell. Absorption spectra were obtained on a Shimadzu UV-2700 Power spectrometer. Fluorescence imaging of HeLa cells was performed with Nikon A1MP confocal microscopy. The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter

3. MTT assay

Cytotoxicity studies were performed using MTT assay. HeLa cells (10^{6} cells / mL) were dispersed within replicate 96-well microtiter plates to a total volume of 200 μL well. Plates were maintained at 37 °C in a 5% CO_2 /95% air incubator for 4 h. **Si-LDs** was diluted to different concentrations of solution with medium and added to each well after the original medium has been removed. HeLa cells were incubated with probe concentrations (0, 1, 5, 10, 20 μM) for 24 h. 200 μL MTT solution (5.0 mg/mL, HEPES) was added to each well. After 4 h, the remaining MTT solution was removed, and 150 μL of DMSO was added to each well to dissolve the formazan crystals. Finally, the absorbance was measured at 520 nm using a microplate reader (Infinite M 200 Pro).

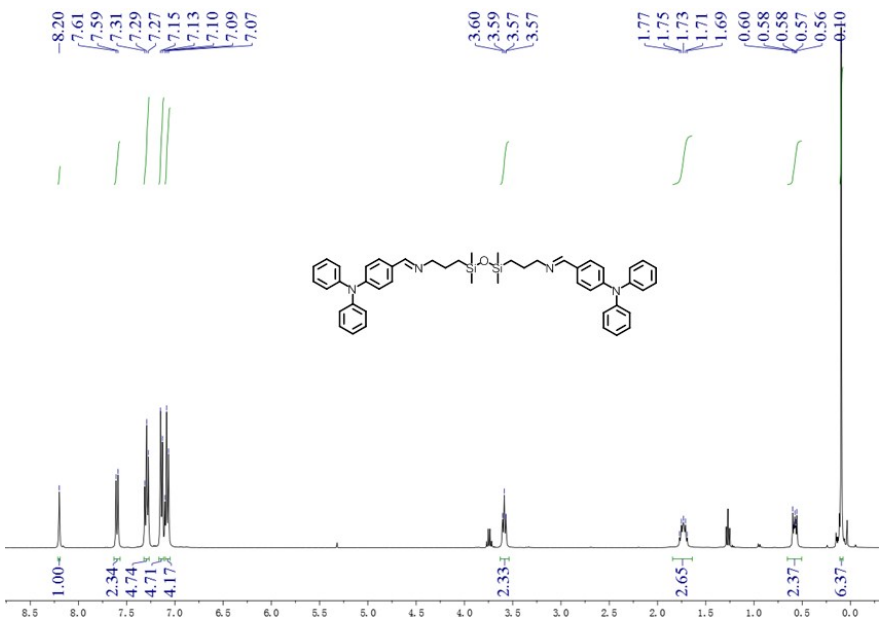

Figure S1. ^{1}H NMR spectrum of **Si-LDS**in $CDCl_3$

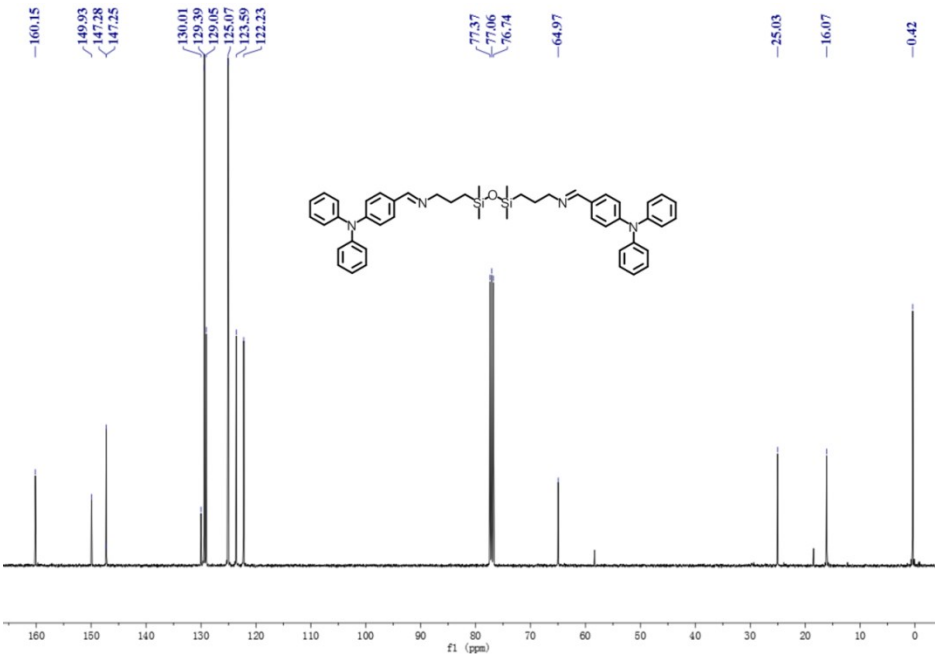

Figure S2. ^{13}C NMR spectrum of **Si-LDS**in $CDCl_3$

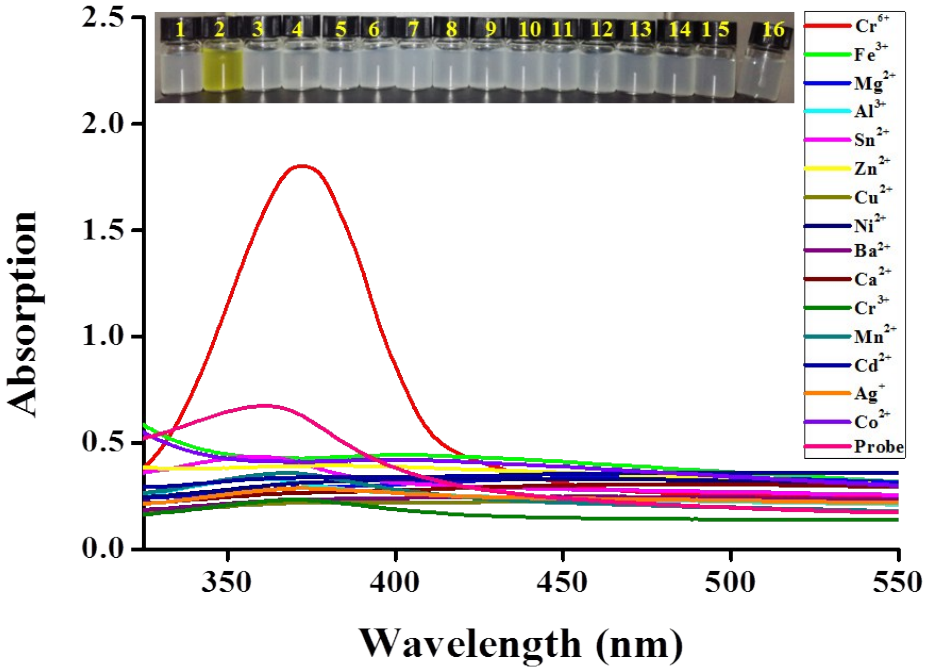

Figure S3. Absorption spectra of **Psi** (10 μM) in the present of different metal ions (1 **Si-LDS** 2 Cr^{6+} 3 Al^{3+} 4 Fe^{3+} 5 Ag^{+} 6 Co^{2+} 7 Sn^{2+} 8 Zn^{2+} 9 Ca^{2+} 10 Mg^{2+} 11 Ba^{2+} 12 Ni^{2+}13 Cr^{3+}14 Mn^{2+} 15 Cd^{2+} 16 Cu^{2+}) (15 equiv.) in an aqueous solution (25 μM PBS buffer, pH = 7.4, containing 10% ethanol).

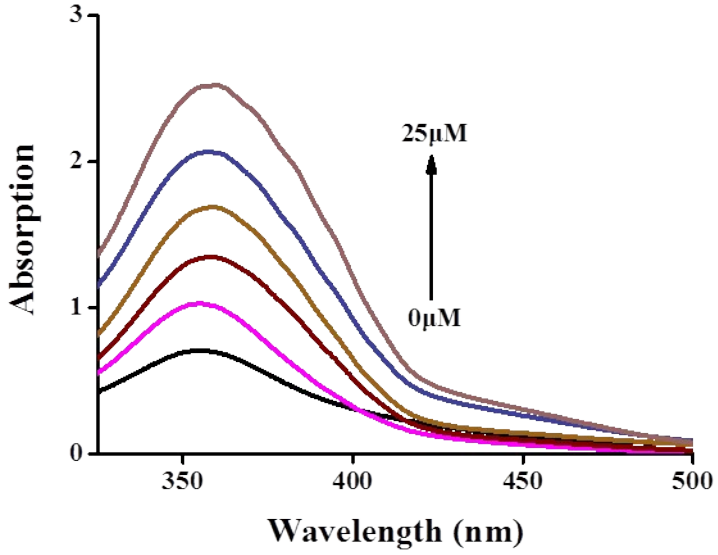

Figure S4. The absorption spectra of **Si-LDS** in aqueous solution (25 μM PBS buffer, pH = 7.4, containing 10% ethanol) in the presence of various contents of Cr^{6+}.

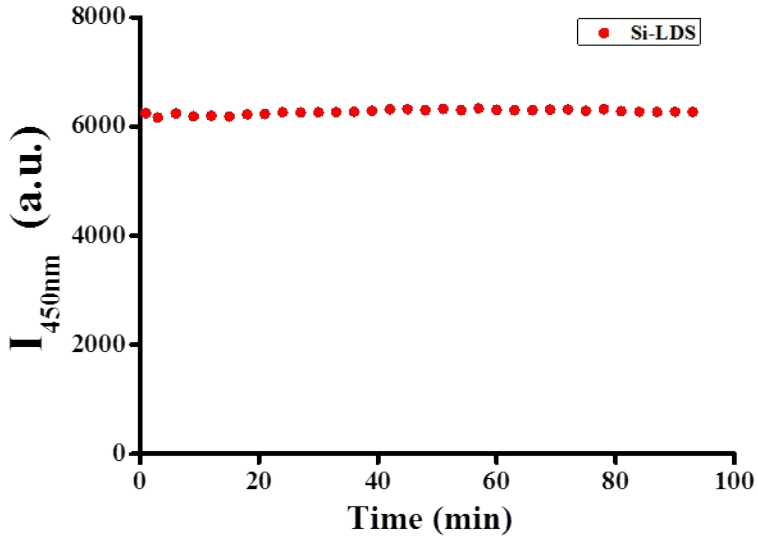

Figure S5. Photo-stability testing result for **Si-LDS** in aqueous solution (25 μM PBS buffer, pH = 7.4, containing 10 % ethanol). Fluorescence was measured at $\lambda_{ex/em}$=365 / 450 nm.

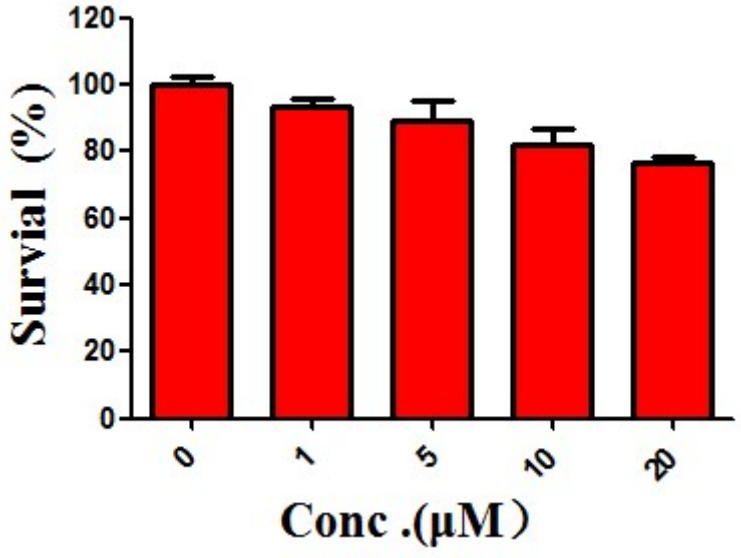

Figure S6. Cytotoxicity of **Si-LDS** on HeLa cells determined by MTT.

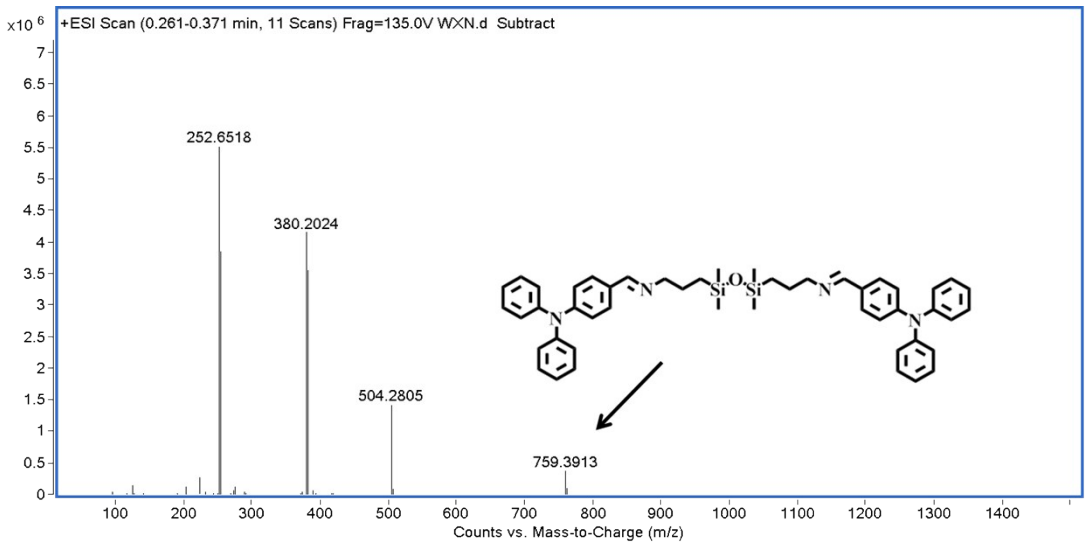

Figure S7. MS spectrum of **Si-LDS** in methanol