Supplementary Material

Polydiacetylene-based colorimetric and fluorometric sensors for lead ion recognition

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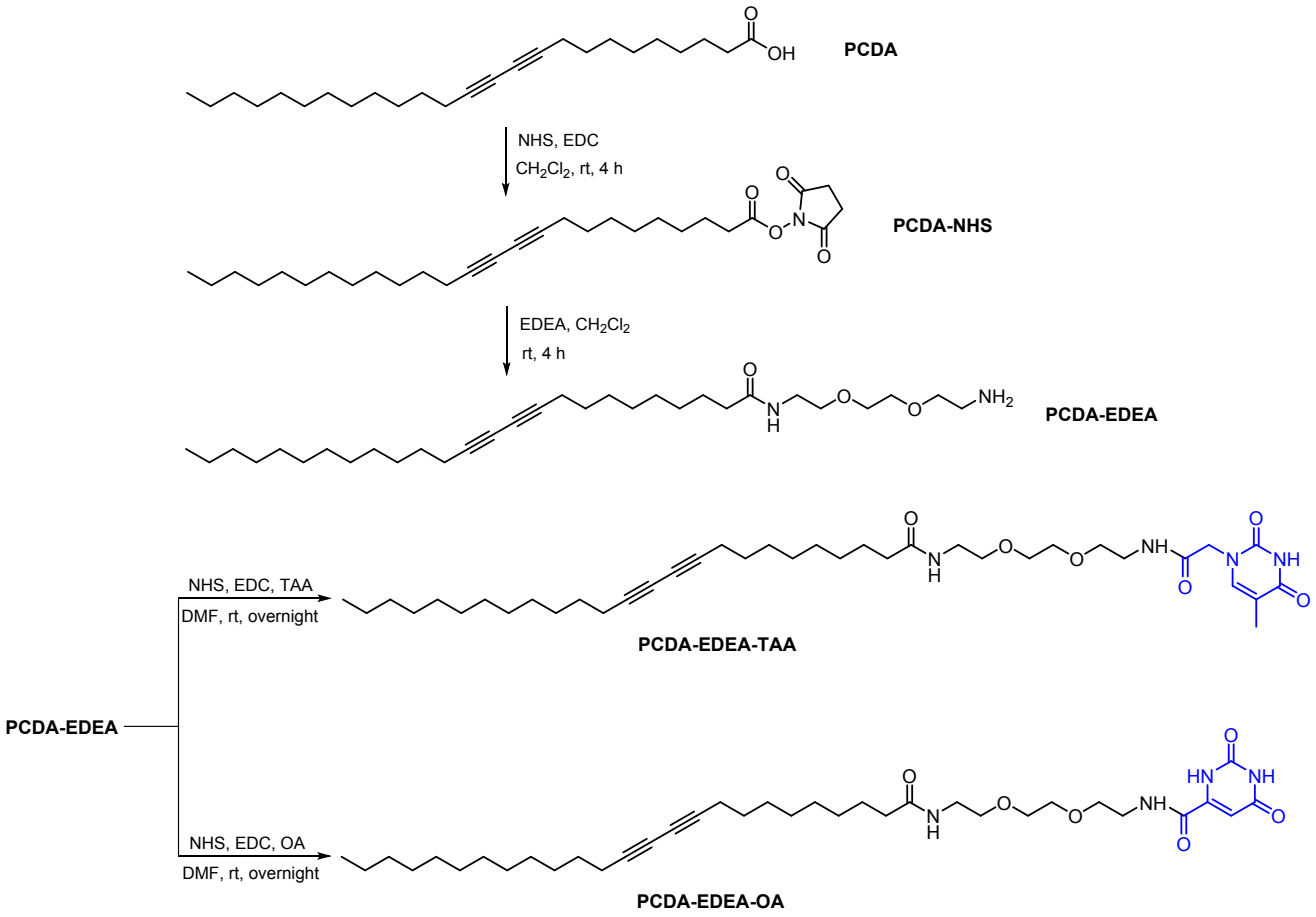
1. Chemicals and instrumentation

10,12-Pentacosadiynoic acid (PCDA) and thymine-1-acetic acid (TAA) were purchased from Alfa Aesar (Lancaster, England). N-Hydroxysuccinimide (NHS) was purchased from Aladdin (Shanghai, China). Orotic acid (OA), 2,2'-(ethylenedioxy)bis(ethylamine) (EDEA) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydro chloride (EDC·HCl) were purchased from Energy Chemical (Shanghai, China). Hydroxyethylpiperazine ethane sulfonic Acid (HEPES) was purchased from Sigma-Aldrich. The PCDA powder was dissolved in dichloromethane and filtered through a 0.45 μm nylon filter to remove oligomers prior to use. All the other reagents or solvents were purchased from local commercial suppliers and used without further purification. The HEPES buffer (10 mM, pH = 7.4) was prepared using the ultrapurified water (18.2 MΩ·cm) from a Milli-Q reference system (Millipore, USA). ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DMX-500 MHz/125 MHz NMR spectrometer (Bruker, Billerica, MA, USA). Chemicals shifts (δ) were specified in ppm relative to Tetramethylsilane (TMS) or solvent residual peaks as internal standards. Electrospray ionization mass spectroscopy (ESI–MS) spectra were recorded by Thermo Scientific LCQ FLEET mass spectrometer (Thermo Fisher Scientific, Waltham, USA). Dynamic light scattering (DLS) was characterized by Zetasizer (Malvern Instruments Co, UK). The pH values were measured with a digital pH meter (PHS-3C, LEICI, Shanghai, China). Transmission electron microscope (TEM) was conducted on HT7700 (HITACHI, JAPAN). The fluorescence emission spectra and UV-Vis absorption spectra were recorded on a Shimadzu RF-5301PC fluorescence spectrometer (Shimadzu, Kyoto, Japan) and a Shimadzu UV1800 spectrometer (Shimadzu, Kyoto, Japan) respectively.

2. Synthesis and characterization

A typical procedure for the preparation of PCDA-EDEA-TAA and PCDA-EDEA-OA is as follows.



Scheme S1. Synthesis procedure of PCDA-EDEA-TAA and PCDA-EDEA-OA

2.1. Synthesis of PCDA-NHS

To a solution of 10,12-pentacosadiynoic acid (PCDA, 213.5 mg, 0.57 mmol) in 20 mL of anhydrous CH_2Cl_2 was added N-Hydroxysuccinimide (NHS, 76.0 mg, 0.66 mmol) and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 138.0 mg, 0.72 mmol) at room temperature. The resulting solution was stirred at room temperature for 4 h, and after evaporation of the solvent in vacuo, the residue was extracted with ethyl acetate 3 times and washed with saturated sodium chloride solution 3 times. Then combined the organic phase, the solvent was removed in vacuo. The

residue was purified by silica column chromatography (CH_2Cl_2/CH_3OH = 20:1, *v*/*v*) to give PCDA-NHS (229 mg, 85.2%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.83 (s, 4H), 2.60 (t, *J* = 7.4 Hz, 2H), 2.24 (t, *J* = 6.8 Hz, 4H), 1.54-1.24 (m, 32H), 0.88 (t, *J* = 7.0 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.18, 168.64, 77.57, 77.45, 65.30, 60.36, 31.92, 30.92, 29.65, 29.63, 29.61, 29.48, 29.35, 29.10, 28.89, 28.86, 28.79, 28.70, 28.37, 28.29, 25.60, 24.55, 22.67, 21.01, 19.20, 19.17, 14.19, 14.10 ppm.

2.2. Synthesis of PCDA-EDEA

To a solution of 2,2'-(Ethylenedioxy)bis(ethylamine) (EDEA, 711.4 mg, 4.8 mmol) in 8 mL of anhydrous CH_2Cl_2 at room temperature, PCDA-NHS (231.1 mg, 0.49 mmol) in 10 mL of anhydrous CH_2Cl_2 was added. The resulting solution was stirred at room temperature for 4 h. After concentration under vacuum, the residue was extracted with CH_2Cl_2 3 times and washed with saturated sodium chloride solution 3 times, then combined the organic phase, the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH = 15:1, *v*/*v*) to afford PCDA-EDEA (192 mg, 78.4%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.33 (s, 1H), 3.63 (s, 4H), 3.59-3.52 (m, 4H), 3.48-3.43 (m, 2H), 2.90 (t, *J* = 5.1 Hz, 2H), 2.24 (t, *J* = 6.8 Hz, 4H), 2.18 (t, *J* = 7.5 Hz, 2H), 1.93 (s, 2H), 1.62 (t, *J* = 6.8 Hz, 2H), 1.55-1.25 (m, 30H), 0.88 (t, *J* = 7.0 Hz, 3H) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.27, 77.58, 77.43, 73.04, 70.26, 70.12, 70.03, 65.32, 65.25, 41.62, 39.13, 36.67, 31.92, 29.64, 29.63, 29.61, 29.48, 29.34, 29.27, 29.21, 29.10, 28.95, 28.86, 28.79, 28.37, 28.32, 25.71, 22.69, 19.21, 19.19, 14.12 ppm.

2.3. Synthesis of PCDA-EDEA-TAA

To a solution of N-Hydroxysuccinimide (NHS, 29.9 mg, 0.26 mmol) and 1-(3-Dimethylamino

propyl)-3-ethylcarbodiimide hydrochloride (EDC, 53.7 mg, 0.28 mmol) in N,N-dimethylformamide (DMF, 20 mL), thymine-1-acetic acid (TAA, 42.4 mg, 0.23 mmol) was added at room temperature. The resulting solution was stirred at room temperature for 3 h, after evaporation of the solvent in vacuo, the residue was extracted with ethyl acetate 3 times and combined the organic phase, then washed with saturated sodium chloride solution. The solvent was removed in vacuo. To the residue in 25 mL of DMF, PCDA-EDEA (116.1 mg, 0.23 mmol) was added. The resulting mixture was stirred at room temperature overnight, and after evaporation of the solvent in vacuo, the residue was extracted with CH_2Cl_2 3 times, combined the organic phase, then washed with saturated sodium chloride solution 3 times. The solvent was removed in vacuo, and the residue was purified by silica column chromatography (CH_2Cl_2/CH_3OH = 10:1, *v*/*v*) to give PCDA-EDEA-TAA (113 mg, 73.2%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d6): δ 11.30 (s, 1H), 8.28 (t, *J* = 5.1 Hz, 1H), 7.87 (t, *J* = 5.1 Hz, 1H), 7.48 (s, 1H), 4.33 (s, 2H), 3.57 (s, 4H), 3.50-3.42 (m, 4H), 3.30-3.22 (m, 4H), 2.32 (t, *J* = 6.8 Hz, 4H), 2.10 (t, *J* = 7.4 Hz, 2H), 1.81 (s, 3H), 1.54-1.45 (m, 6H), 1.37-1.25 (m, 26H), 0.91 (t, *J* = 7.0 Hz, 3H) ppm; ^{13}C NMR (125 MHz, DMSO-d6): δ 172.71, 167.47, 164.94, 151.49, 142.89, 108.37, 79.67, 78.43, 70.08, 70.01, 69.68, 69.45, 65.84, 49.73, 39.26, 38.92, 35.78, 31.80, 29.50, 29.45, 29.36, 29.20, 29.16, 29.11, 28.89, 28.72, 28.66, 28.24, 28.20, 25.73, 22.59, 18.77, 14.43, 12.38 ppm. HRMS (ESI-MS): calcd. for $C_{38}H_{63}N_4O_6$ $[M + H]^+$ 671.4748, found 671.4735.

2.4. Synthesis of PCDA-EDEA-OA

To a solution of N-Hydroxysuccinimide (NHS, 26.5 mg, 0.23 mmol) and 1-(3-Dimethylamino propyl)-3-ethylcarbodiimide hydrochloride (EDC, 47.9 mg, 0.25 mmol) in 20 mL of DMF, orotic acid (OA, 35.9 mg, 0.23 mmol) was added at room temperature. The resulting solution was stirred at room

temperature for 2 h. Then PCDA-EDEA (116.1 mg, 0.23 mmol) in DMF (15 mL) was added to the above solution. The resulting mixture was stirred at room temperature for 6 h. DMF was removed in vacuo, the residue was extracted with CH_2Cl_2 3 times, combined the organic phase and washed with saturated sodium chloride solution 3 times. The solvent was removed in vacuo, and the residue was purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH = 10:1, *v*/*v*) to give PCDA-EDEA-OA (42 mg, 28.4%) as a white solid. HRMS (ESI-MS): calcd. for $C_{38}H_{63}N_4O_6Na$ [M + Na]$^+$ 665.8718, found 665.4246.

3. ^{1}H NMR, ^{13}C NMR and HRMS spectra

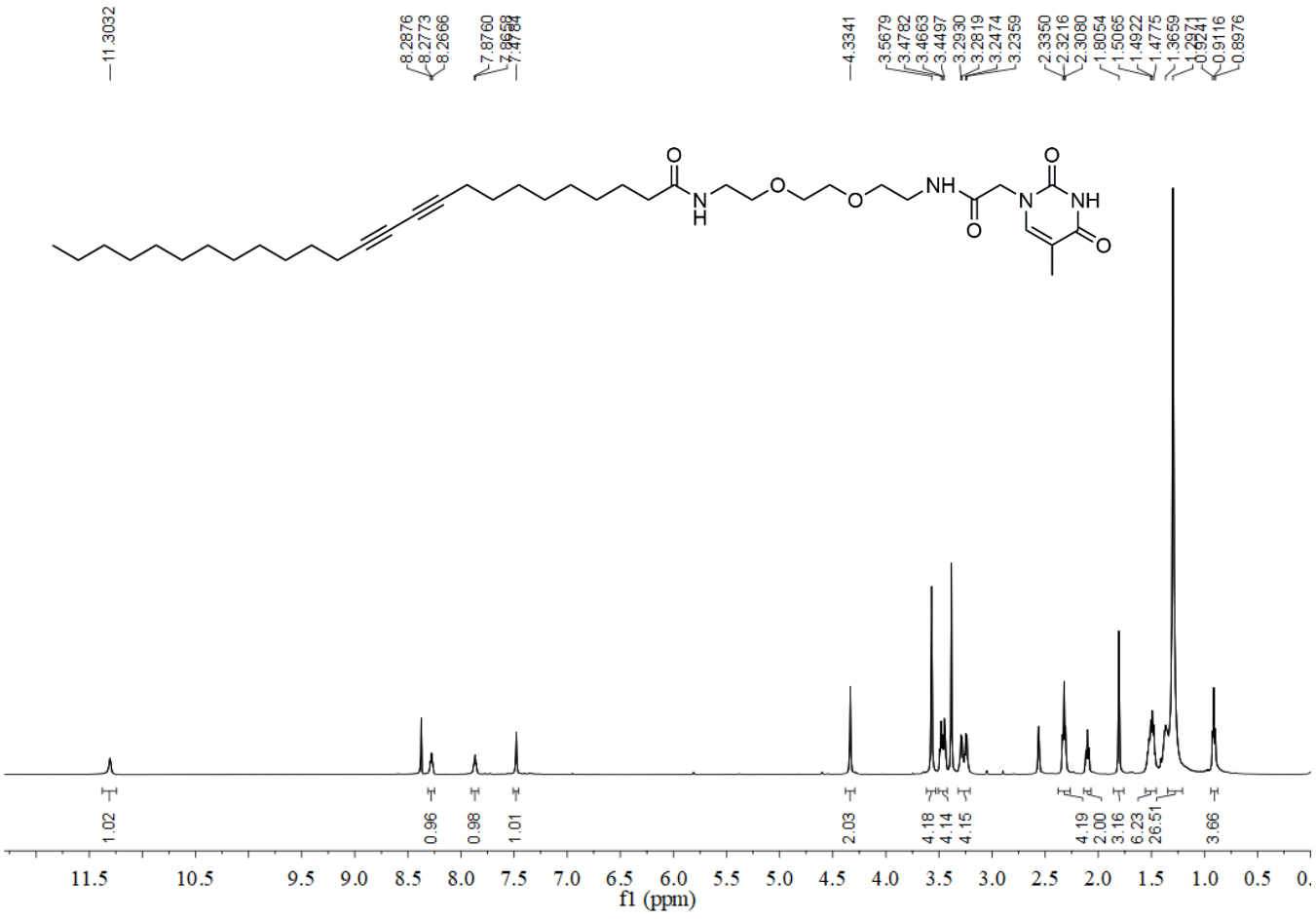


Fig. S1. ^{1}H NMR spectrum of PCDA-EDEA-TAA in DMSO-d6 (500 MHz).

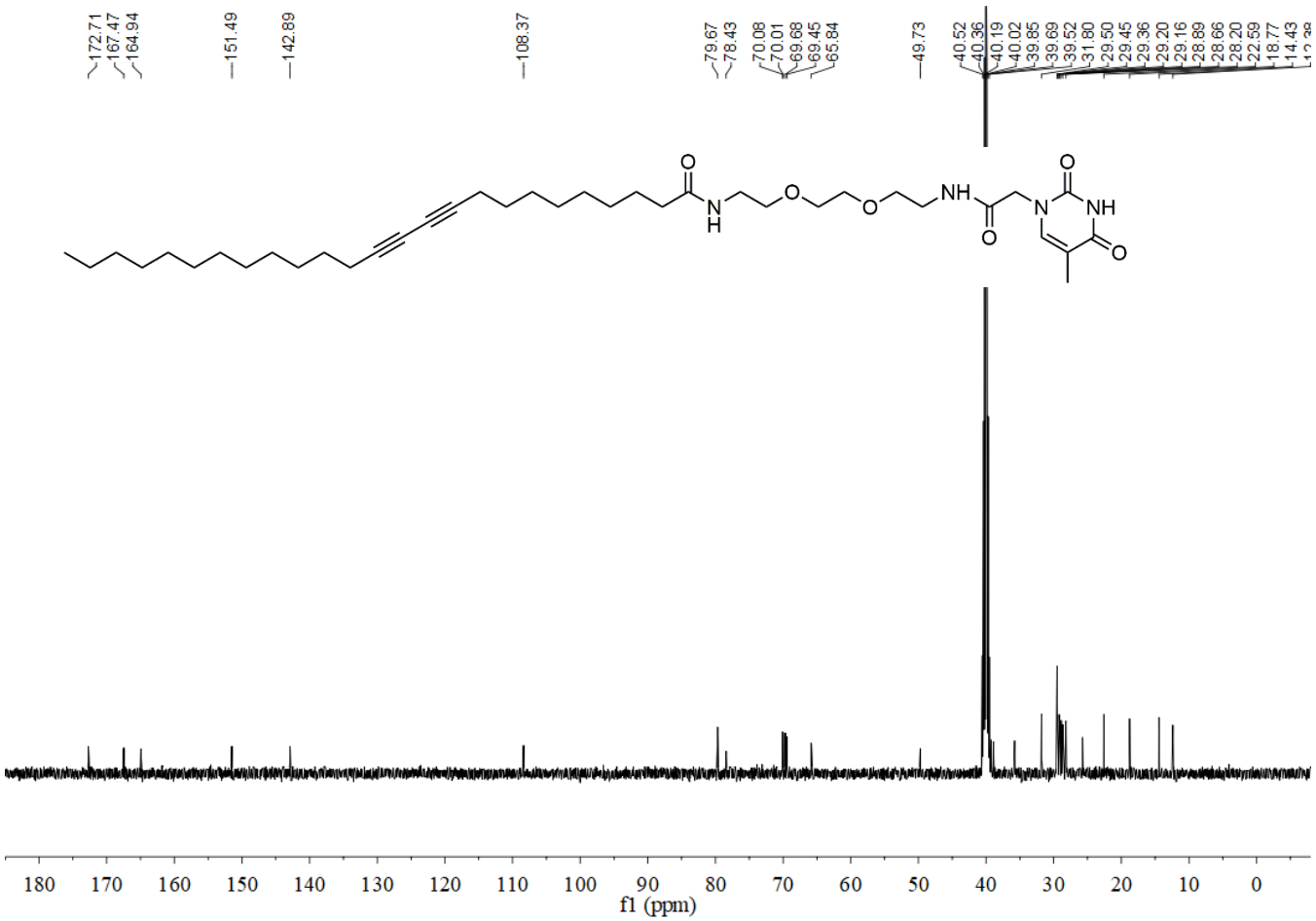


Fig. S2. ^{13}C NMR spectrum of PCDA-EDEA-TAA in DMSO-d6 (125 MHZ).

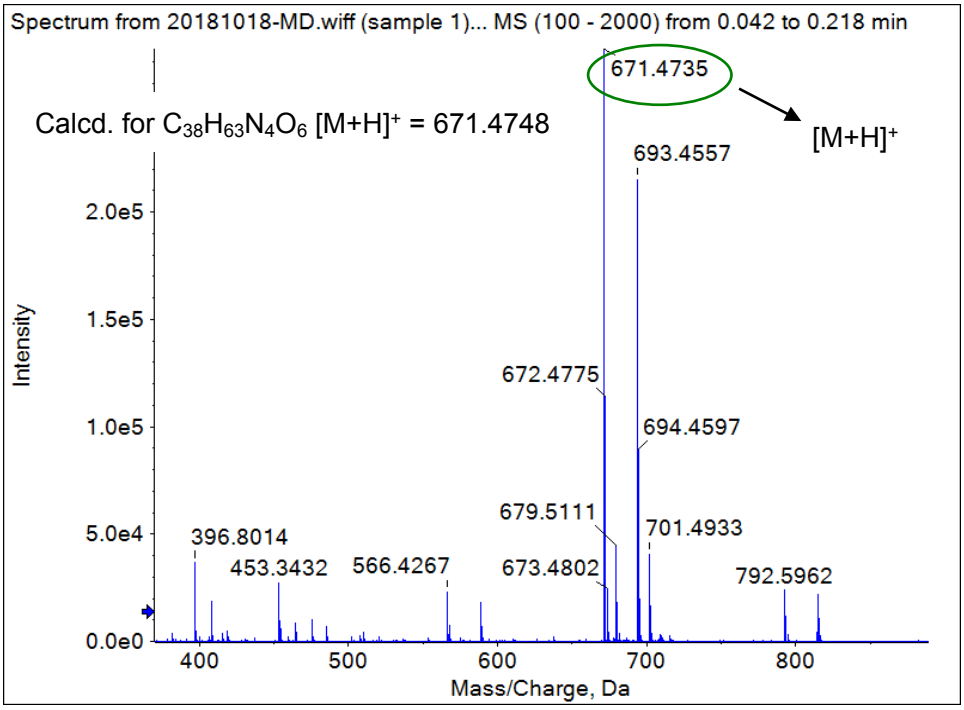


Fig. S3. ESI-Mass spectrum of PCDA-EDEA-TAA (ESI-MS).

4. Study on the size distribution of PDA-EDEA-TAA liposomes by DLS.

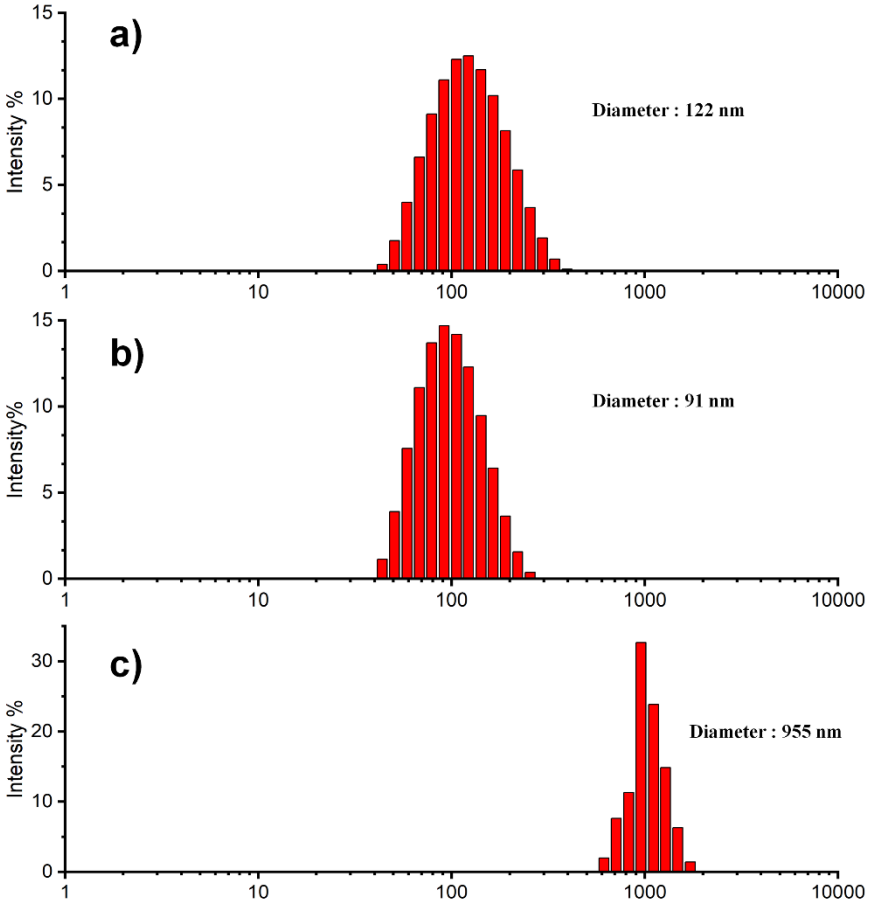


Fig. S4. Particle size distribution of PDA-EDEA-TAA liposomes measured by DLS in HEPES (10 mM, pH = 7.4): (a) before and (b) after UV irradiation (254 nm), (c) in the presence of 100 μM Pb^{2+}.

5. *In situ* ^{1}H-NMR spectroscopy studies of PCDA and PCDA-EDEA-TAA in the presence of Pb^{2+}.

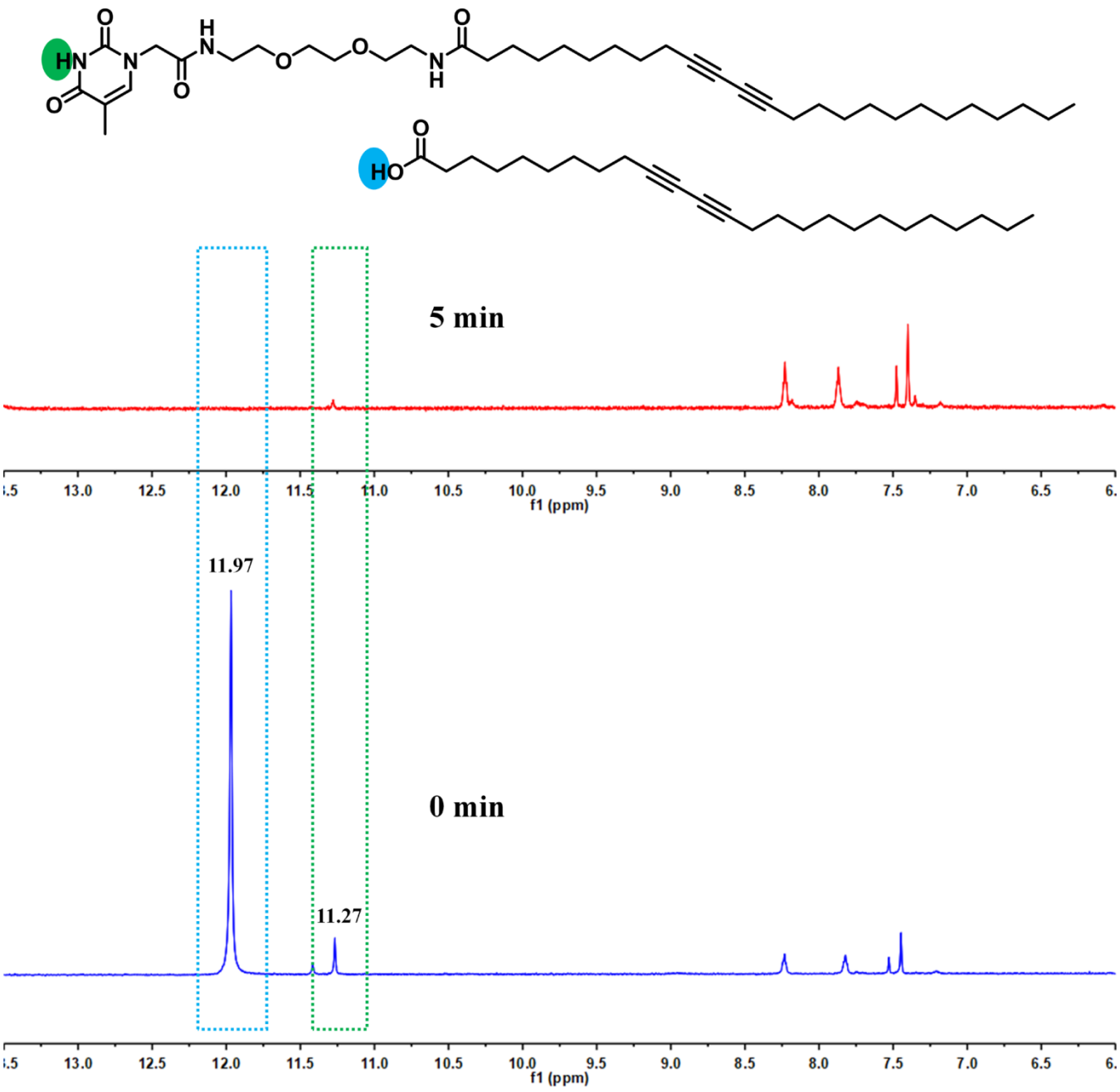


Fig. S5. The partial ^{1}H-NMR spectra (DMSO-*d6*, 500 MHz) of the mixture containing PCDA and PCDA-EDEA-TAA (mole ratio = 9:1) after the addition of 100 μM Pb^{2+}.

6. Study on the response time of PDA-EDEA-TAA liposomes for Pb^{2+}.

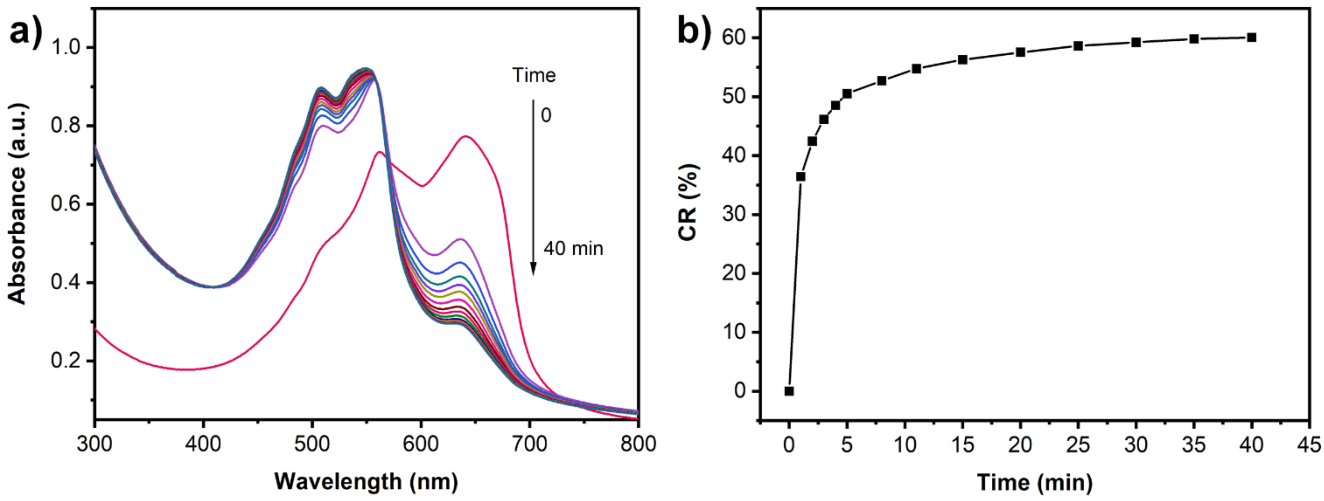


Fig. S6. (a) UV-vis absorbance spectra of PDA-EDEA-TAA liposomes (100 μM) upon the addition of Pb^{2+} (100 μM) as a function of time. (b) Corresponding CR (%) value changes of PDA-EDEA-TAA liposomes upon the addition of Pb^{2+} with increasing time.

7. Study on changes in UV-vis absorption spectra and fluorescence emission spectra of PDA-EDEA-OA liposomes with the increasing concentration of Pb^{2+}.

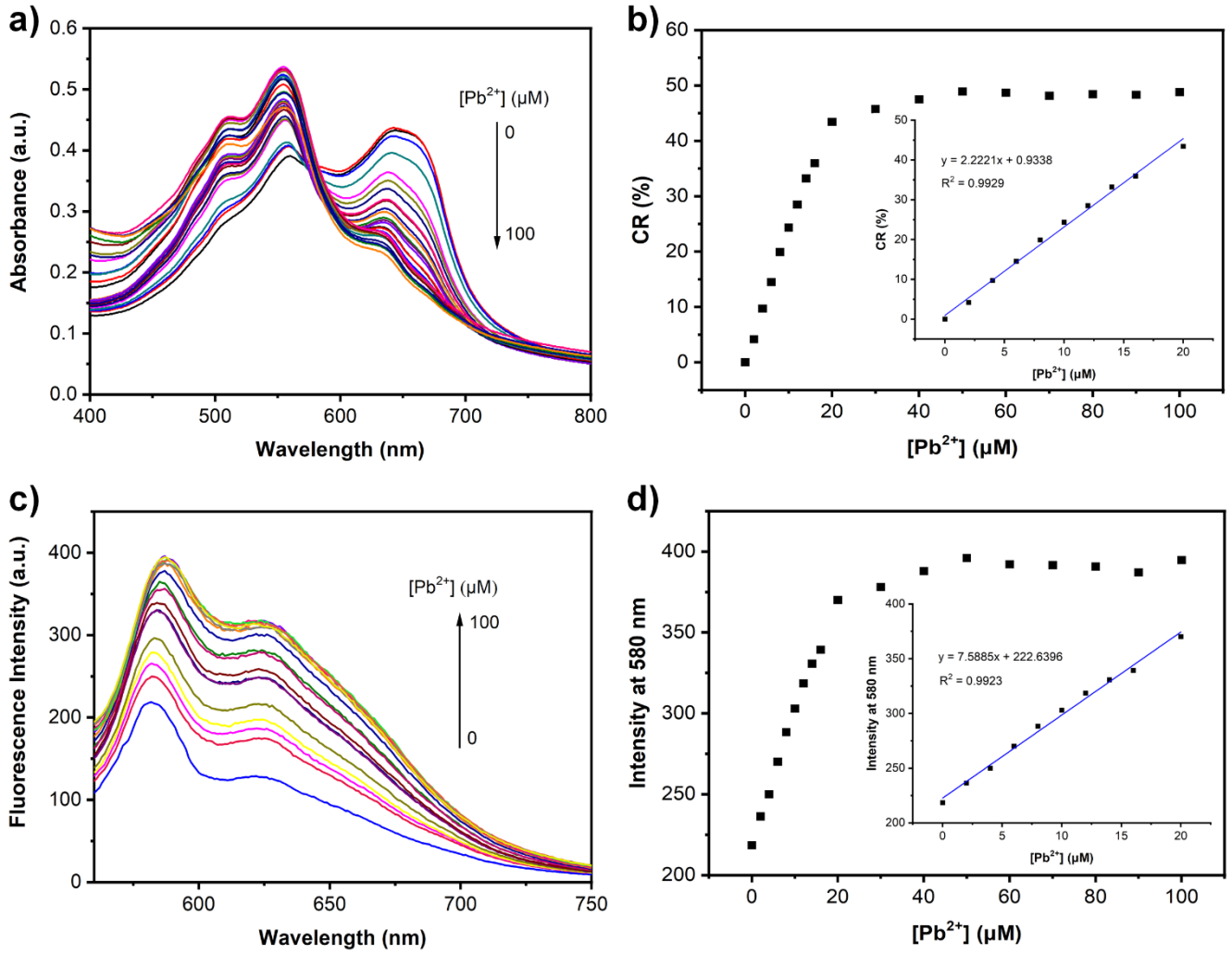


Fig. S7. (a) UV-vis absorption spectra and (b) related CR (%) values of PDA-EDEA-OA liposomes (100 μM) in HEPES buffer (10 mM, pH = 7.4) with the increasing concentration of Pb^{2+} ions (0-100 μM) at room temperature. Inset: the linear relationship between the CR (%) value of PDA-EDEA-OA liposomes and Pb^{2+} concentrations. (c) Fluorescence emission spectra and (d) related fluorescence intensity changes at 580 nm of PDA-EDEA-OA liposomes (100 μM) in HEPES buffer (10 mM, pH = 7.4) upon addition of different concentrations of Pb^{2+} (0-100 μM) at room temperature. Inset: the linear relationship between the fluorescence intensity of PDA-EDEA-OA liposomes and Pb^{2+} concentrations.

8. Study on the selectivity of PDA-EDEA-OA liposomes for Pb^{2+}.

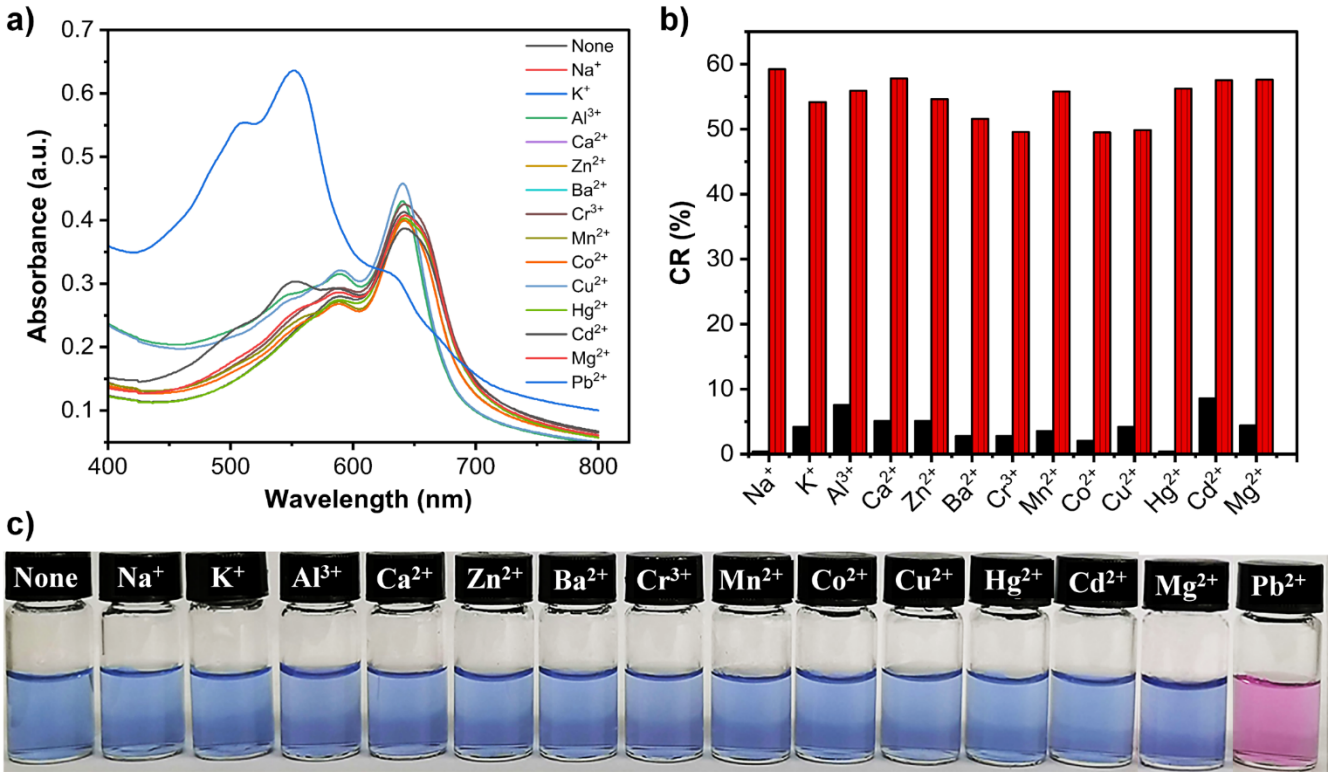


Fig. S8. (a) UV-Vis absorption spectra and (b) Related CR (%) values of PDA-EDEA-OA liposomes in the presence of different metal ions. Black bars represent the CR (%) values after the addition of the given metal ions (100 μM). Red bars represent the CR (%) values after the addition of Pb^{2+} ions (100 μM) to the respective solution. (c) The color changes of PDA-EDEA-OA liposomes (100 μM) upon the addition of different metal ions in HEPES buffer (10 mM, pH = 7.4) at room temperature.

9. Study on the selectivity of PDA liposomes prepared from pure PCDA.

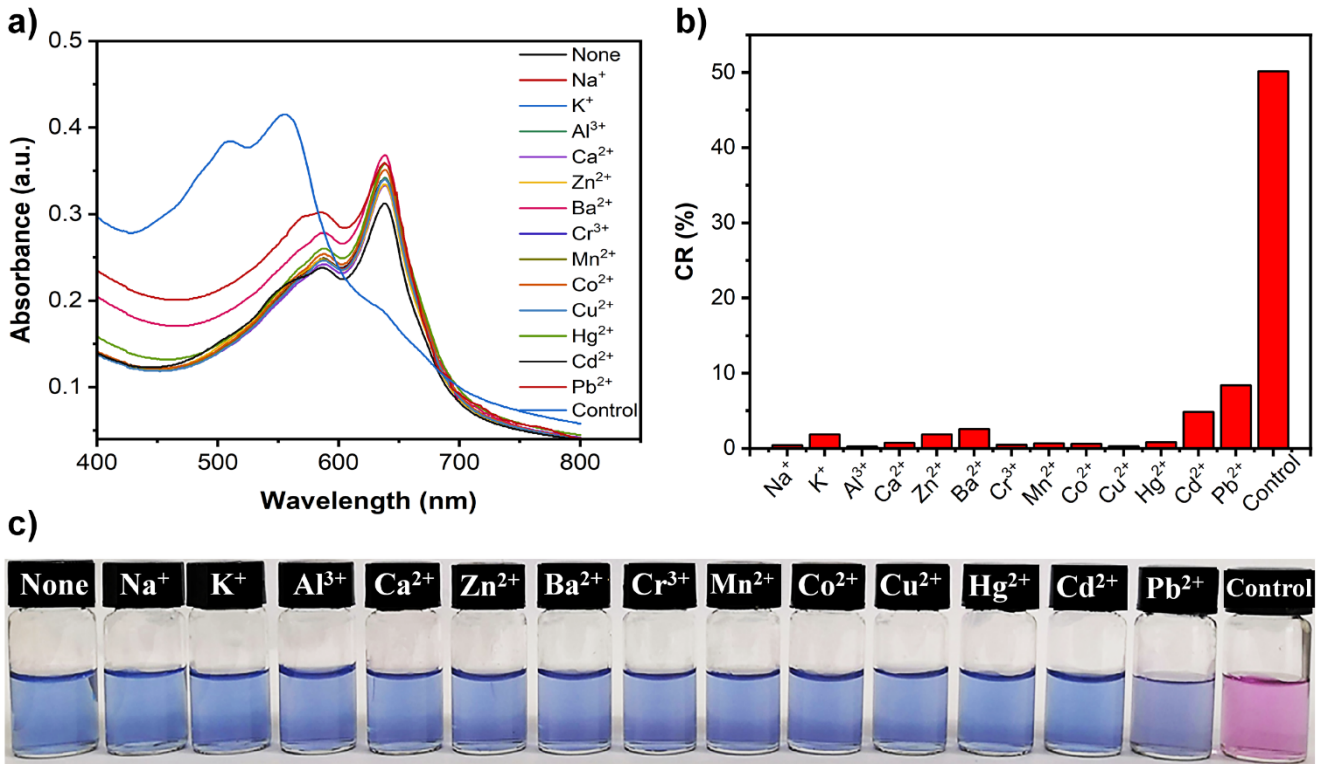


Fig. S9. (a) UV-vis spectra and (b) related CR (%) values of PDA liposomes prepared from pure PCDA in HEPES (10 mM, pH = 7.4) in the presence of different metal ions (100 μM) in HEPES buffer (10 mM, pH = 7.4) at room temperature. (c) Corresponding color changes of PDA liposomes prepared from pure PCDA after adding different metal ions. Control group was set as the CR (%) value and color change of PDA-EDEA-OA liposomes in the presence of Pb^{2+} (100 μM).

10. Study on the binding constants (*K*).

Table S1. The binding constants (*K*).

Metal ions	Pb^{2+}	Ag^{+}	Hg^{2+}	Mg^{2+}	Ca^{2+}	Mn^{2+}	Cd^{2+}	Cu^{2+}	Ba^{2+}	Co^{2+}	Cr^{3+}	Na^{+}	K^{+}
K	7.2×10^{4}	6.3×10^{4}	4.6×10^{4}	3.8×10^{4}	3.5×10^{4}	3.0×10^{4}	2.6×10^{4}	2.4×10^{4}	2.0×10^{4}	1.6×10^{4}	1.0×10^{4}	6.8×10^{3}	6.7×10^{3}

11. Study on the selectivity coefficients (K).

Table S2. The selectivity coefficients (K).[a]

Metal ions	Na^{+}	K^{+}	Mg^{2+}	Al^{3+}	Ag^{+}	Cu^{2+}	Ba^{2+}	Co^{2+}	Cr^{3+}	Fe^{3+}	Hg^{2+}	Ca^{2+}	Cd^{2+}	Mn^{2+}
Slopes	0.0142	0.0079	0.0184	0.3629	0.0204	0.0466	0.0190	0.0238	0.0838	0.2336	0.0410	0.0214	0.2058	0.0138
K	0.6%	0.3%	0.8%	15%	0.8%	2.0%	0.8%	1.0%	3.6%	10%	1.8%	0.9%	8.9%	0.8%

[a]The selectivity coefficient is the ratio of the slope of given metal ions to the slope of Pb^{2+}, the slope of Pb^{2+} is 2.2948.