

Polydiacetylene Vesicles Functionalized with N-Heterocyclic Ligands for Metal Cation Binding

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1. General methods and material

Absorption Spectroscopy. Absorption were recorded on a Varian Cary BIO 50 UV/VIS/NIR Spectrometer with temperature control by use of a 1 cm quartz cuvettes (Hellma) and aqueous buffered solution (HEPES 10 mmol, pH = 7.2).

Emission Spectroscopy. Fluorescence measurements were performed with aqueous buffered solution (HEPES 10 mmol, pH = 7.2) in 1 cm quartz cuvettes (Hellma) and recorded on a Varian 'Cary Eclipse' fluorescence spectrophotometer with temperature control.

NMR Spectra. Bruker Avance 600 (1H: 600.1 MHz, 13C: 150.1 MHz, T = 300 K), Bruker Avance 400 (1H: 400.1 MHz, 13C: 100.6 MHz, T = 300 K), Bruker Avance 300 (1H: 300.1 MHz, 13C: 75.5 MHz, T = 300 K). The chemical shifts are reported in δ [ppm] relative to external standards (solvent residual peak). The spectra were analysed by first order, the coupling constants are given in Hertz [Hz]. Characterisation of the signals: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bs = broad singlet, psq = pseudo quintet, dd = double doublet, dt = double triplet, ddd = double double doublet. Integration is determined as the relative number of atoms. Error of reported values: chemical shift: 0.01 ppm for 1H-NMR, 0.1 ppm for 13C-NMR and 0.1 Hz for coupling constants. The solvent used is reported for each spectrum.

Mass Spectra. Varian CH-5 (EI), Finnigan MAT 95 (CI; FAB and FD), Finnigan MAT TSQ 7000 (ESI). Xenon serves as the ionisation gas for FAB.

IR Spectra. Recorded with a Bio-Rad FTS 2000 MX FT-IR and Bio-Rad FT-IR FTS 155.

Melting Point. Melting Points were determined on Büchi SMP or a Lambda Photometrics OptiMelt MPA 100.

Dynamic light scattering (DLS). Dynamic light scattering (DLS) for particle size measurements were performed by using Zetasizer 3000 from Malvern instruments Ltd. Malvern, UK using 1 cm UV-visible cuvettes. Vesicle solutions were diluted 5 to 6-fold and measured at RT by keeping the count rate at 80-100 kcps. Each diameter value was an average result of continuous measurements over 5 min. At least three measurements were performed for each solution.

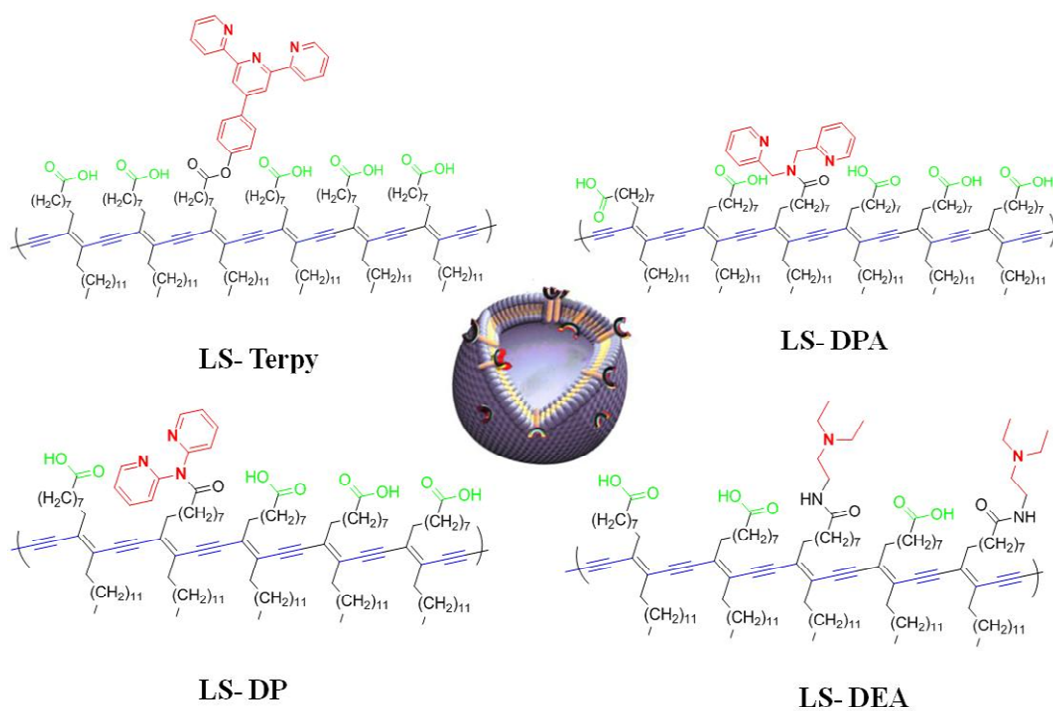
2. Binding studies

General: All binding studies were conducted in buffered aqueous solution (HEPES 10 mmol, pH 7.2). The cuvette with 3000 mL of liposomes in HEPES buffered solution was titrated stepwise with small amounts (beginning with 0.2 equiv) of the cation solution. Chloride salts of Zn^{2+} , Hg^{2+} , Ni^{2+} , Cu^{2+} , Co^{2+} , Cr^{2+} , Hg^{2+} , Mn^{2+} , Mg^{2+} , Ca^{2+} and nitrate salts of Cd^{2+} , K^+ , Na^+ , Pb^{2+} , Li^+ and Ag^+ were used for the binding studies. After each addition the solution was allowed to equilibrate five minutes before the fluorescence and the UV spectrum (where permitted by the concentration range) were recorded. The total amount of metal ion required for saturation was determined by plotting the graph between the changes in absorbance at 540 nm against the concentration of added metal ions. To determine the binding constants, the obtained absorbance at 540 nm was volume corrected, plotted against the metal ion concentration and evaluated by nonlinear curve fitting.

UV-absorption titration: Stock solutions (10 mL) of the liposomes **LS-Terpy** (1.0×10^{-3} M), **LS-DPY** (1×10^{-3} M), **LS-DP** (1×10^{-3} M) and **LS-DEA** (1.0×10^{-3} M) were prepared in buffered aqueous solution (HEPES 10 mmol, pH = 7.2), respectively, and stored in dark. These solutions were appropriately diluted and irradiated by UV light before the spectroscopic studies. Solutions of the respective anions (100 mmol) were prepared and were stored in the dark. All titration experiments were performed using 1.0×10^{-5} M solutions of **LS-Terpy**, **LS-DPY**, **LS-DP** and 5.0×10^{-5} M solution of **LS-DEA** in water (HEPES 10 mmol, pH 7.2) and various concentrations of the metal ions (1×10^{-5} - 1×10^{-3} M) in the same solvent. Based on the absorption spectral changes the colorimetric response (CR) was derived by using the following equation as mentioned in manuscript. $\% \text{ CR} = [(A_0 - A_x)/A_0] \times 100$

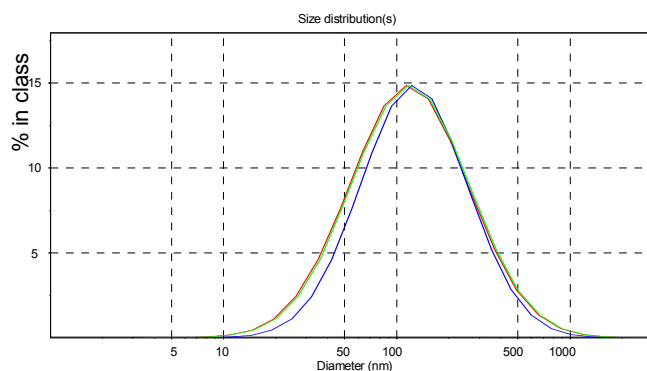
The CR value is derived from the change in the ratio of absorbance at 640 nm and 550 nm in the absence (A_0) and presence (A_x) of different analytes. The absorption ratio before analyte addition is calculated as $A_0 = I_{640} / (I_{640} + I_{550})$ and the absorption ratio after analyte addition follows from $A_x = I_{640} / (I_{640} + I_{550})$, respectively.

Emission titration: The standard solutions mentioned above were used for emission titration studies. For all measurements the liposome solutions were excited at 510 nm, with an excitation and emission slit width of 10 nm.

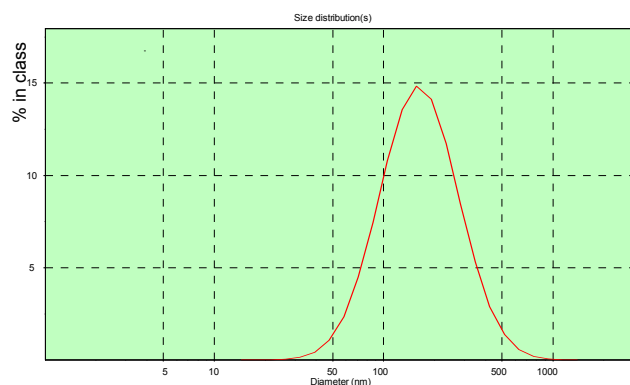


SI Figure 1: Different surface modified self-assembled vesicular receptors

3. Particle size distribution curves.



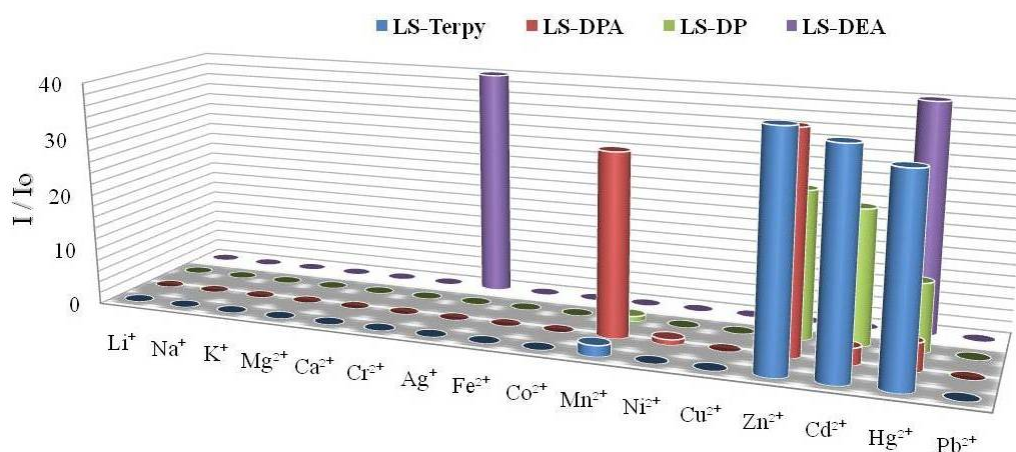
SI Figure 2a: DLS curves of the **LS-DP** (HEPES, pH=7.2). Three different colours indicate three different measurements of the same solution.



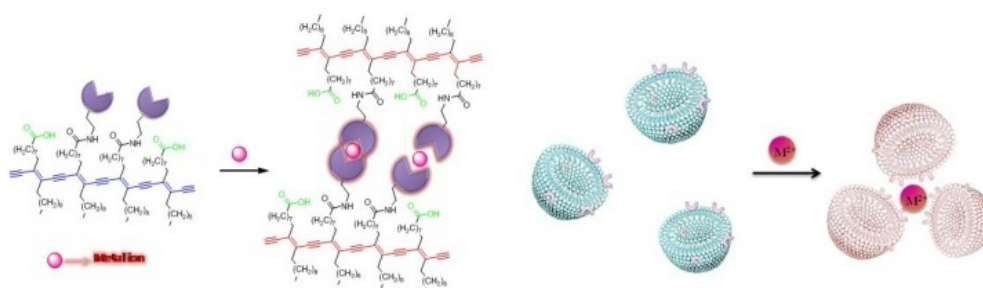
DLS curves of the **LS-DPA** (HEPES, pH=7.2).

SI Figure 2b: Example of Particle size distribution curves

4. Changes of the vesicle emission intensity



SI Figure 3: Changes of the vesicle emission intensity upon addition of different metal cations



SI Figure 4: Schematic representation of metal ion binding with ionophore-modified vesicular receptors

5. ¹H NMR and ¹³C spectra of synthesized compounds

