Polydiacetylene vesicles functionalized with N-heterocyclic ligands for metal cation binding†

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Received 8th September 2009, Accepted 31st October 2009
First published as an Advance Article on the web 10th December 2009
DOI: 10.1039/b918452j

Self assembled poly diacetylene (PDA) based blue vesicles **LS-Terpy**, **LS-DPA**, **LS-DP** and **LS-DEA** with metal chelating sites have been prepared and characterized. Their response to the presence of metal cations in buffered aqueous solution has been investigated by monitoring changes of colour, UV-Vis absorption and emission. The addition of zinc, manganese, cadmium, mercury or silver salts to solutions of the vesicles induces a colour change from blue to red observable by the naked eye, while the addition of other metal salts, containing ions like Li^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Cr^{2+}, Ni^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+} or Pb^{2+}, failed to show any changes. The metal ion coordination selectivity of the ligands is slightly different for the vesicle surface immobilized ligands compared to the reported metal cation binding of the corresponding ligands in solution, which may be due to the special environment at the lipid–solution interface. The vesicles aggregate upon metal ion coordination to the embedded ligands as shown by dynamic light scattering (DLS) particle size analysis. The functionalized PDA vesicles retain their response to the presence of aqueous solutions of metal ions if immobilized in transparent polyvinyl alcohol films or on paper.

Introduction

Vesicular particles have been employed in diverse applications in biological research, due to their relative ease of preparation and variability in composition.[1] Interactions of hosts and guests at vesicle surfaces resemble recognition processes at biological membranes, including cell recognition, adhesion and fusion. Synthetic receptor sites have been incorporated into self-assembled monolayers or bilayer membranes,[2] leading to extended functionalized interfaces, which are able to interact with guest molecules or ions from solution. Well defined self-assembly processes starting from rather simple building blocks allow their easy preparation and molecular recognition events in the special environment of the lipid–solution interface may benefit from increased affinities or altered selectivities compared to homogeneous solution.[3] Polydiacetylene (PDA) is a particularly interesting material in this respect, as diacetylene surfactants self assemble in water to form vesicles that can be photopolymerized to generate PDA *in situ*. PDA exhibits an intense colour change from blue to red in response to external stimuli such as temperature, pH, ions, solvent or mechanical stress.[4] The colour change is visible by the naked eye and easily monitored by UV absorption spectroscopy. This unique colour transition has been widely used to monitor binding processes at interfaces.[5] Recently, we described receptor modified PDA vesicles that respond to the presence of biologically important anions like ATP and PPi.[6]

The selective coordination of metal ions to vesicles bearing suitable amphiphilic ligands has been reported and in some cases, it results in the formation of unusual complexes and remarkable changes in the lateral organization of the membrane.[7] However, only a few reports describe the use of PDA based materials for the recognition of cations at the vesicular interface,[4b,8] and the examples are limited to alkali and alkali earth metal cations. Thus we have prepared amphiphilic diacetylenes with N-heterocyclic metal chelating head groups,[9] which are suitable for the coordination of transition metal cations, such as Zn^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+}. The functionalized amphiphilic diacetylenes were self-assembled into vesicles and photopolymerized to give PDA particles exhibiting transition metal cation affinity. The composition of the vesicles was altered and their metal cation binding selectivity was determined to reveal how their properties can be modulated by varying the embedded ligands.

Result and discussion

Terpyridine (**3**, Terpy), di-(2-picolyl) amine (**4**, DPA) and dipyridine (**5**, DP) were used as N-heterocyclic head groups of 10, 12-pentacosodiyonic acid (**1**, PCDA) derived amphiphiles. A derivative of diethyl amine (**6**, DEA) was prepared for comparison (Fig. 1). The N-heterocyclic ligands are well known to form defined complexes with many transition metal cations and have been widely used for the preparation of coordination compounds and molecular sensors.[10] However, in many cases complex formation requires non-aqueous conditions.

Synthesis of PCDA monomers

The diacetylene amphiphiles **7**, **8**, **9** and **10** were prepared by amide or ester formation as shown in Scheme 1. Compound **7** was prepared by reacting **2** with **3** in dry THF under ice

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† Electronic supplementary information (ESI) available: General methods and material, ^1H and ^{13}C NMR spectra for new compounds, binding studies and particle size distribution plots. See DOI: 10.1039/b918452j

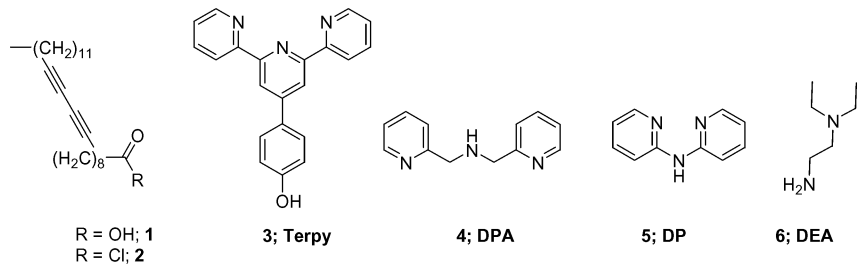
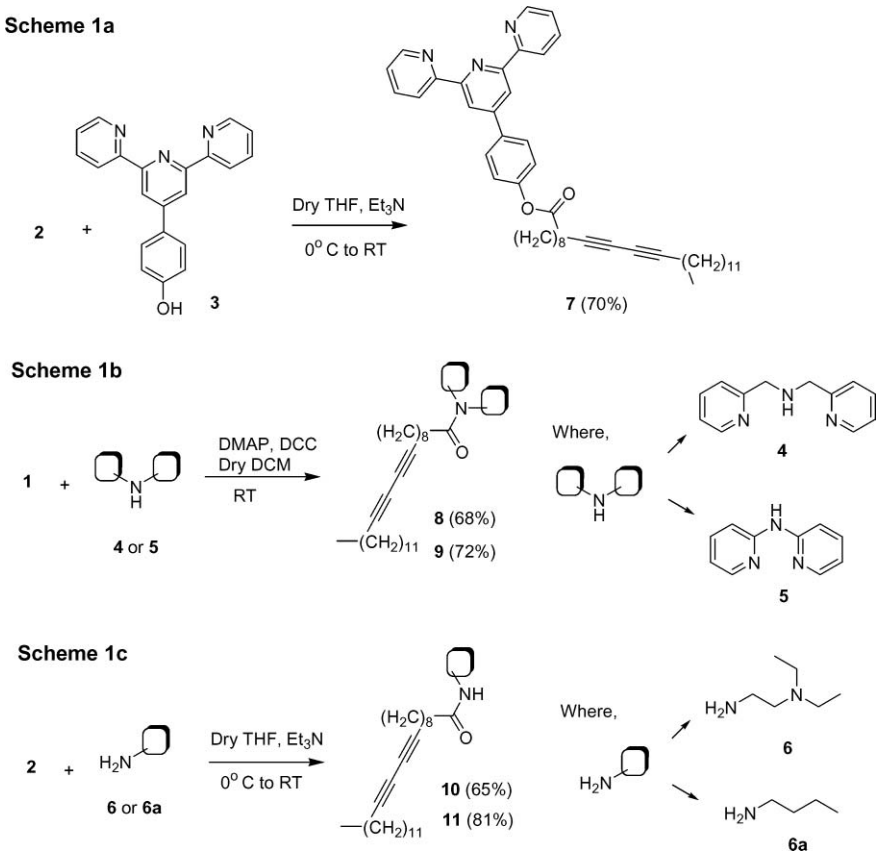


Fig. 1 Structures of the ligands used as head groups for diacetylene amphiphiles.



Scheme 1 General synthesis of receptor modified amphiphilic diacetylene monomers.

cooling (Scheme 1a). Compounds **8** and **9** were prepared at room temperature starting from compound **1** using DMAP and DCC in dry DCM with good yields (Scheme 1b). Compound **10** was prepared by amide formation at 0 °C and subsequent reflux at 75 °C (Scheme 1c). All diacetylene amphiphiles **7**, **8**, **9** and **10** were characterized by standard analytical and spectroscopic techniques and are very stable when stored under nitrogen in the dark. Detailed experimental procedures and analytical data of the compounds are provided in the experimental section.

Vesicle preparation

A liposome solution containing unmodified 10,12-pentacosadiynoic acid (**1**, PCDA) and receptor modified PCDA *i.e.* **7**, **8**, **9**, and **10** was prepared using an earlier reported procedure.[5,11] According to that, a mixture containing the diacetylene amphiphiles **7**, **8**, **9** or **10** and diacetylene carboxylic acid **1** in a molar ratio of 10 : 90, respectively, was dissolved in dichloromethane in a 25 mL round bottom flask. The solvent was evaporated by a stream of N_2 gas and an appropriate amount of buffered aqueous solution (HEPES 10 mmol, pH = 7.2) was added to the flask to give the desired concentration of the lipid (1×10^{-3} M). This solution was sonicated at 75–80 °C for 40 min. The resulting milky solution was filtered through a syringe filter (pore size: 1 μm, filter: 15 mm) while hot, the filtrate was cooled and stored at 0 °C overnight. The self-assembled vesicles were polymerized at room temperature by irradiating the solutions with light of 254 nm wavelength for 5 to 10 min, whereby the colourless vesicle solution turned blue. The average size of the liposomes was 150–250 nm as determined by dynamic light scattering (DLS). The stability of the vesicles depends on the concentration of the vesicles. Highly concentrated vesicle solutions are unstable and the vesicles will precipitate over time. More diluted samples are very stable when stored at 4 °C (> 3 month test period). Liposomes containing **7** are named

Table 1 Particle size of the prepared vesicles determined by dynamic light scattering

Vesicles	Particle size in nm
LS-Terpy	250 ± 10
LS-DPA	190 ± 10
LS-DP	120 ± 10
LS-DEA	135 ± 10

LS-Terpy, liposomes containing **8** are named **LS-DPA**, liposomes containing **9** are named **LS-DP**, and liposomes containing **10** are named **LS-DEA**, respectively (Fig. 1, ESI†). The metal ion binding of the blue colour nanometre sized vesicles in solution was monitored by UV-visible and emission spectroscopy. The concentration of the final liposome solution was determined following a literature described method.[12]

Particle size measurement

Dynamic light scattering (DLS) particle sizing measurements were performed by a Zetasizer 3000 from Malvern instruments Ltd. Malvern, UK. Vesicle solutions were diluted 5- to 6-fold and measured at room temperature keeping a typical count rate at 30–50 kcps. Each diameter value is an average result of continuous measurements over 5 min. At least three measurements were performed for each solution. The measured particle size values are summarized in Table 1. The obtained values are in good agreement with comparable liposomes that have been reported earlier in literature.[13] The two-component vesicles have an average size of 250 nm. Upon UV irradiation, the vesicle size shrinks to 160–200 nm (Fig. 2, ESI†).

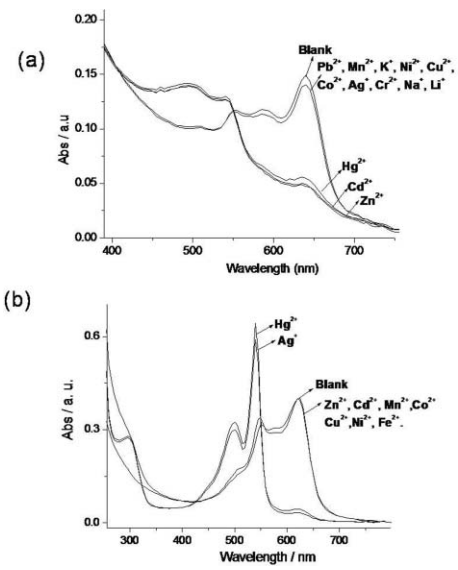


Fig. 2 Change in absorption spectra of (a) **LS-Terpy** (1×10^{-5} M) and (b) **LS-DEA** (5×10^{-5} M) with different metal ions in aqueous buffer solution. Chloride salts of Zn^{2+}, Hg^{2+}, Ni^{2+}, Cu^{2+}, Co^{2+}, Cr^{2+}, Mn^{2+}, Mg^{2+}, Ca^{2+} and nitrate salts of Cd^{2+}, K^{+}, Na^{+}, Pb^{2+}, Li^{+}, Ag^{+} used for binding studies.

Metal ion binding

The metal ion binding properties of the vesicles **LS-Terpy**, **LS-DPA**, **LS-DP** and **LS-DEA** were examined for several alkali, alkaline earth and transition metal cations in buffered water (HEPES, pH = 7.2, 10 mmol L^{-1}).

Absorption spectra of 5×10^{-5} M solutions of the blue coloured vesicles in the absence and the presence of the metal salts were recorded. The absorption spectra of the different functionalized liposomes in the absence of any metal ion show absorption bands at 640 and 589 nm. The colour response and the spectral change of the functionalized liposomes was screened adding different alkali, alkaline earth or transition metal ions like Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Cr^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Hg^{2+}, Cd^{2+}, Ag^{2+}, Zn^{2+} and Pb^{2+}. Only in a few cases the absorption band at 640 nm completely disappeared, the colour of the solution became red and the intensity of the absorption at 589 and 543 nm increased. (Fig. 2). Fig. 3 shows the visible colour changes induced by the different metal cations added to an aqueous solution of the functionalized vesicles and demonstrates that the blue to red colour transition of the vesicles depends on the nature of metal cation.

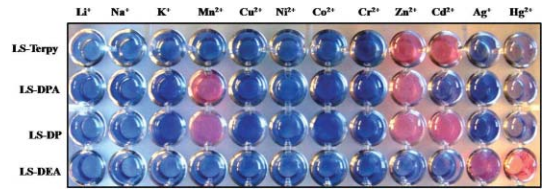


Fig. 3 Colour change of functionalized vesicle solutions (1×10^{-4} M) in water (HEPES buffer, pH = 7.2) upon addition of different metal salts (1×10^{-3} M) at room temperature. The solutions were allowed to equilibrate for 5 min after the salt addition.

The colour change from blue to red was quantified by calculating the colorimetric response (**CR**, Table 2).[5,14] A systematic change in the UV-Vis spectra of **LS-Terpy**, **LS-DPA**, **LS-DP** and **LS-DEA** associated with varying concentration of different metal ions are shown in Fig. 4. Spectral and colour change reveal that **LS-Terpy** has a slightly higher affinity for Zn^{2+} ions in water than for Cd^{2+}, Cu^{2+}, Mn^{2+} and Hg^{2+} ions (Fig. 4); terpyridine-type ligands are known to form stable complexes with zinc ions in the solid state.[15] In vesicle solution Zn^{2+} ions displayed a fast response time (~1 min) with a detection limit of 2.1×10^{-6} M, which allows the colorimetric detection of submillimolar concentrations of Zn^{2+} ions. Other metal ions like Cd^{2+} and Hg^{2+} exhibit a slower response kinetic and a full colour change is attained in approximately 3–5 min. The order of metal ion selectivity is $Zn^{2+} > Cd^{2+} > Hg^{2+} \gg Cu^{2+}$ or Ni^{2+}. The metal ion binding properties for **LS-Terpy** are consistent with the previously reported results for simple terpyridine ligands.

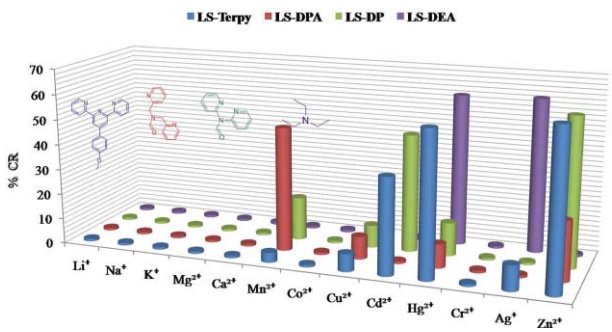


Fig. 4 Comparative plot of the colorimetric response of receptors with metals.

Table 2 Colorimetric response (%**CR**) [a]of the receptors with different metal ions

Receptor	Cu^{2+}	Cd^{2+}	Mn^{2+}	Zn^{2+}	Ni^{2+}	Hg^{2+}	Ag^{+}	Fe^{2+}
LS-Terpy	7	38	4	62.1	10	57.2	10	ND[b]
LS-DPA	9	—	49.1	53.5	—	9.5	—	—
LS-DP	9	46.6	17.2	58.8	—	13.2	—	—
LS-DEA	—	—	—	—	—	60.1	61	—

[a] Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Cr^{2+}, Co^{2+} and Pb^{2+} ions did not show any spectral changes to obtain **CR** values. [b] not determined due to high level of precipitation.

Table 3 Apparent binding constant (log K_{app}) [a]values of the metal ion vesicular receptors

Metal ion affinity (log K_{app})								
Receptor	Cu^{2+}	Cd^{2+}	Mn^{2+}	Zn^{2+}	Ni^{2+}	Fe^{2+}	Hg^{2+}	Ag^{+}
LS-Terpy	<1.0	5.1	—	5.8	<1.0	—[b]	3.4	—
LS-DPA	—	<1.0	5.5	4.3	—	—	3.1	—
LS-DP	—	6.2	—	6.1	—	—	3.2	—
LS-DEA	—	—	—	—	—	—	2.3	2.2

[a] Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Cr^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+} and Pb^{2+} ions did not show any significant spectral changes for calculating binding constant. [b] Not determined due to high level of precipitation.

However, contrary to a recent report,[16] which shows preferable Hg^{2+} binding for a terpyridine derivative in DMSO/water (1 : 3.5) solution, our vesicle immobilized terpyridine ligand displays only weak interactions with Hg^{2+} ions and strong binding of Zn^{2+} and Cd^{2+} ions. Terpyridine ligands are known to form manganese complexes in solution,[17] but at the vesicular PDA interface it failed to coordinate Mn^{2+} ions.

DPA ligands[18] show nanomolar affinity to zinc ions at neutral pH in the presence of high concentrations of other metal cations.[19] **LS-DPA** discriminates manganese and zinc ions with apparent log K_{app} values of 5.5 and 4.3. The metal cation selectivity observed for **LS-DPA** (Table 3) is consistent with the established binding affinities of the **DPA** ligands. Ligand **5** (**DP**) is known to coordinate with various metals ions in solution, like Mg^{2+}, Ca^{2+}, Sr^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, or Zn^{2+} under various conditions.[20] However, embedded into a vesicle (**LS-DP**) the ligand responds only to zinc and cadmium ions in water with nanomolar affinity (Fig. 4). The metal ion binding selectivity is obviously affected by the conditions at the lipid–solution interface.

LS-DEA shows a response to the presence of mercury or silver ions (Table 3) at millimolar concentrations.[21] Amide and urea NH groups have been reported to interact with metal ions like Hg^{2+}.[22] To investigate the role of the NH group in **LS-DEA** for ion binding, we synthesized the butyl-modified compound **11** for comparison. Interestingly, vesicles prepared using compound **11** did not show any significant spectral change with any metal ions tested. This observation indicates that the amide NH groups are not significant for the observed metal ion binding and the diethyl amine moiety acts as a binding site for mercury and silver ions. This illustrates that the coordination ability of ligands may change significantly at the lipid-solution interface and a simple *N*,*N*-diethyl amino ligand showing only weak interactions with metal ions in solution phase can lead to strong ion responses at the vesicle interface.

Apparent binding constant (log K_{app}) values of the vesicular receptors were derived based on the changes in the absorption spectra with respect to different concentration of metal salts (Fig. 5, Fig. 6, Table 3) by using non-linear regression analysis and assuming a 1 : 1 ligand to metal ion binding at the interface of the vesicles.[11,23]

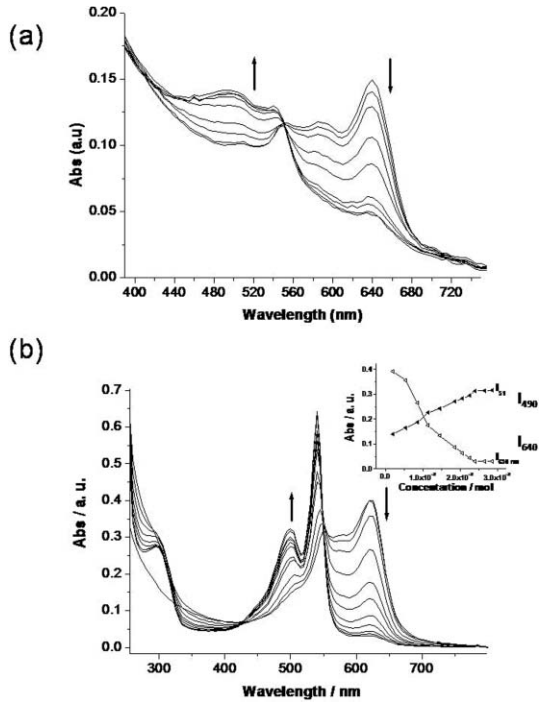


Fig. 5 UV-visible titration of (a) **LS-Terpy** (1×10^{-5} M) with zinc ions (1×10^{-6} M–2×10^{-5} M) (b) **LS-DEA** (5×10^{-5} M) with mercury ions (1×10^{-6} M–5×10^{-2} M) in water, inset: change of absorbance maximum at 610 nm and 510 nm with respect to added Hg^{2+} ion.

Zinc ions lead to an immediate change in UV-Vis absorption with all receptors except **LS-DEA**. Manganese ions induce changes only with receptor **LS-DPA** and with a slow response time of 30 min to reach maximum colour change. Cadmium ions do not induce spectral changes with receptors **LS-DPA** and **LS-DEA**, but interact with **LS-Terpy** and **LS-DP** receptors. Mercury ions give spectral changes in aqueous solution with all receptors, which is strongest in the case of **LS-DEA**. We have also conducted control binding experiments with PDA vesicles that were not modified with metal ion coordinating groups. No changes in the absorption spectra and colour of the solution were observed upon the addition of metal ions. This confirms that the PDA vesicles modification with metal ion binding ligands is responsible for the spectral and colour change of the solution

EDTA-addition experiments were conducted to examine the reversibility of the receptor-metal ion binding event. When EDTA was added to the red solution of vesicles and metal ions the spectral change is not reverted to its original blue form. This result indicates that the metal ion induced colour change of the vesicles from blue to red is not reversible. Irreversible response behaviour of different sensors based on PDA is very common in literature.[24] In a control experiment, we have confirmed that EDTA does not interact with the vesicles in the absence of any metals. The colour transitions of PDA assemblies have been attributed

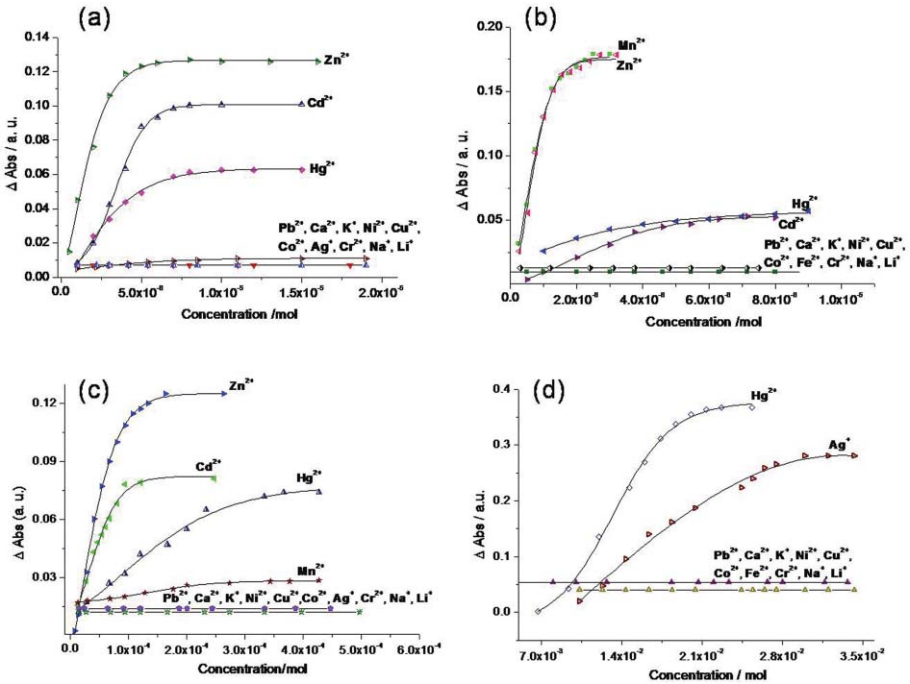


Fig. 6 Titration curves of (a) **LS-Terpy**, (b) **LS-DPA**, (c) **LS-DP** and (d) **LS-DEA** with different metals in water at room temperature.

to conformational transitions in the conjugated (ene-yne) PDA backbone, induced by structural perturbations.[25,26]

Emission studies

Next, changes in the emission properties of the PDA vesicles in response to the presence of metal cations were studied. Emission spectra of the receptors **LS-Terpy**, **LS-DPA**, **LS-DP** and **LS-DEA** (5×10^{-5} M in 10 mM HEPES buffer, pH 7.2) show a very weak emission band centred at 625 nm upon excitation at 510 nm (Fig. 7).

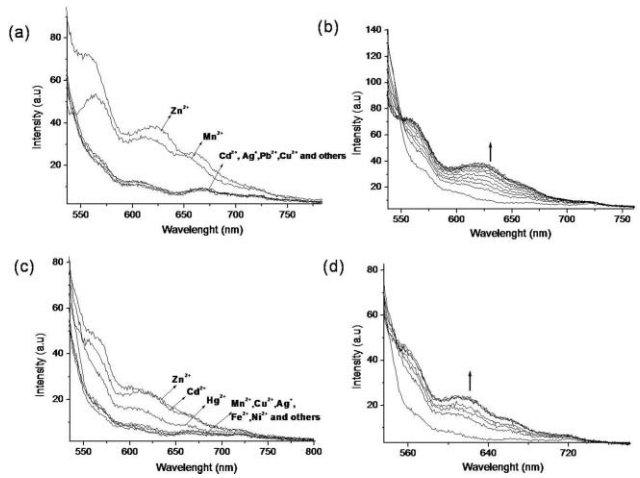


Fig. 7 Emission titration of (a) **LS-DPA** (5×10^{-5} M) with different metal cations (1×10^{-3} M), (b) emission titration of **LS-DPA** (5×10^{-5} M) with manganese ions (1×10^{-6} M–5×10^{-3} M), (c) **LS-DP** (5×10^{-5} M) with different cations (1×10^{-3} M), (d) emission titration of **LS-DP** (5×10^{-5} M) with zinc ions(1×10^{-6} M–5×10^{-3} M).

Upon addition of metal ions (metal ions which responded colorimetrically: Zn^{2+}, Mn^{2+}, Cd^{2+}, Hg^{2+} and Ag^{+}) the emission intensity centred at 600 nm increases about 20-fold. Other ions like Li^{+}, Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, Cr^{2+}, Ni^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+} and Pb^{2+} induced very little or no change in the emission intensity (Fig. 7) upon addition to the vesicle solution. The relative emission intensities of the vesicles with different metal cations are shown in Fig. 3, ESI.†

Based on the observed spectral changes, the response of the functionalized vesicles to the presence of metal cations can be summarized as follows: **LS-Terpy** responds to the presence of zinc and cadmium ions at nanomolar concentrations affinity, but fails to respond to manganese ions as observed by others for terpyridine ligands in homogeneous solutions. **LS-DP** is selective for zinc and cadmium ions. **LS-DPA** responds to the presence of zinc and manganese ions with high affinity, which is in accordance to the properties of **DPA** ligands in solution. **LS-DEA** selectively responds to the presence of mercury and silver ions, which is typically not observed for **DEA** ligands in solution. Overall, the incorporation of the ligands into the vesicles changes their metal ion coordination ability: Different selectivities and increased affinities compared to solution are observed.

Effect of metal ions on vesicular particle size

A solution of **LS-DP** (1×10^{-4} M) was titrated with zinc ions, and the size of the particles was measured after each addition. Upon increasing amounts of zinc ions the observed size of the **LS-DP** particles increased from 120 nm to ~2800 nm (Fig. 8) indicating a metal ion induced aggregation.[7e,27] The size of the vesicular particles is not affected upon addition of non-binding metal ions, such as manganese ions. The experiments reveal that the aggregation depends on the metal ion binding to the interfaces

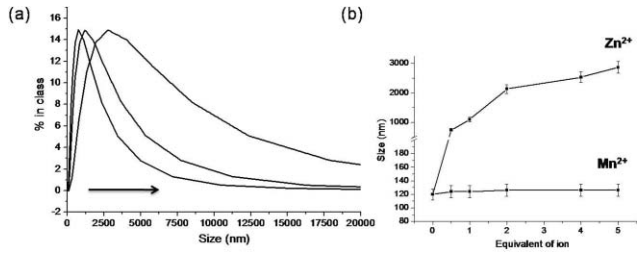


Fig. 8 (a) DLS curves upon addition of Zn^{2+} metal ion in to the **LS-DP** (HEPES, pH = 7.2). (b) Particle size measurement of **LS-DP** with Mn^{2+} and Zn^{2+} ions.

of the vesicles. A schematic representation of the process is shown in Fig. 4, ESI.†

Vesicle immobilization in polyvinyl alcohol films and on paper

To investigate if the response of the functionalized vesicles to metal cations is retained upon immobilization, transparent polyvinyl alcohol films and coated papers with the polymerized vesicular receptor were prepared. The blue coloured solution containing functionalized vesicles was mixed with an aqueous 10 wt% polyvinyl alcohol (PVA) solution (2 ml, 1 : 1, v/v%)[28] and slowly poured in a Petri dish to form a uniform layer.

After drying at room temperature for 36 h a thin film was formed in the Petri dish. This blue-coloured transparent film was removed from the dish (Fig. 9a) and used for binding studies. The metal ion recognition property of the blue colour films was checked with different metal ions. Films prepared using **LS-DPA** were dipped into an aqueous solution containing zinc ions and within the few minutes the colour of the film changed to red. However, the film collapsed due to the water solubility of PVA films (Fig. 9b). In the presence of non-binding metal ions the film colour remains blue (Fig. 9c). To immobilize the functionalized vesicles on water insoluble support, we coated papers with different vesicular receptor following an earlier reported procedure.[6] The obtained test papers are stable in aqueous solution. The colorimetric response of **LS-Terpy**, **LS-DPA**, **LS-DP** and **LS-DEA** coated papers was examined in the presence of different metal ions. The

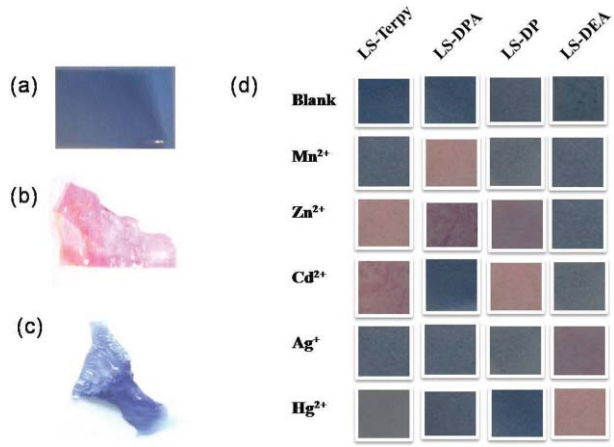


Fig. 9 (a) Transparent blue colour PVA film with embedded **LS-DPA** receptor (b) PVA film with embedded **LS-DPA** receptor in the presence of zinc ions (c) with cadmium ions in water (d) test papers prepared from different vesicular solutions in presence and absence of metal ions.

colour change of the test papers from blue to red indicates the binding of ions to the immobilized vesicle receptors (Fig. 9d).

Conclusions

A series of self assembled polydiacetylene vesicles **LS-Terpy**, **LS-DPA**, **LS-DP** and **LS-DEA** has been prepared. The vesicles display N-heterocyclic ligands at their surfaces and are stable in solution for a long time if stored at 4 °C. Upon addition of zinc, cadmium, manganese, silver or mercury salts to aqueous solutions of the vesicles a visible colour change is observed. Metal complex formation at the interface of the PDA vesicles leads to an aggregation of the vesicles as confirmed by monitoring the particle size. The vesicles retain their binding properties in transparent PVA films and immobilized on paper.

The results show that coordination properties of typical N-heterocyclic ligands can be transferred onto PDA vesicles by embedded co-monomers bearing the ligands, but their specific coordination ability changes at the lipid-solution interface. The method is easily applied to almost any ligand and may find applications in the development of analytical nanoparticles or metal complex immobilization.

Experimental section

Thin layer chromatography (TLC) analyses were performed on silica gel 60 F-254 with a 0.2 mm layer thickness; compound detection by UV light at 254 nm/366 nm or ninhydrin staining in EtOH. Column chromatography was performed on silica gel (70–230 mesh) from Merck. Starting materials were purchased from either Acros or Sigma-Aldrich and used without any further purification. Commercially available solvents of standard quality were used. Dry THF, which was prepared by distillation from potassium. If otherwise stated, purification and drying was done according to accepted general procedures.[29]

Synthesis and characterization of compounds

Compounds **2**[30] and **3**[31] were synthesised according to known literature procedures. Other compounds are prepared by the following synthetic protocols with good yield and purity.

Pentacosa-10,12-diynoic acid 4-[2,2′;6′,2″]terpyridin-4′-yl-phenyl ester (7). Compound **3** (100 mg, 0.31 mmol) was dissolved in dry THF under N_2 atmosphere and compound **2** (122 mg, 0.32 mmol) and 0.25 mL of Et_3N were added. This reaction mixture was stirred at 0 °C overnight then the reaction mixture was heated to reflux for 1 h. The white coloured salt formed was filtered and the clear solution was evaporated to dryness. The crude product was purified by silica column chromatography using ethyl acetate and petroleum ether as the eluent. The desired product **7** was collected as the first fraction in the form of colourless solid. **MP**: 95–98 °C, **Yield**: 170 mg (75%). ^{1}**H-NMR** (300 MHz, $CDCl_3$): δ (ppm) = 0.87 (t, ^3J = 7.1 Hz, 3 H, CH_3), 1.21 (m, 26 H, CH_2), 1.51 (t, ^3J = 7.5 Hz, 4 H, CH_2), 1.77 (t, ^3J = 7.4 Hz, 2 H, CH_2), 2.25 (m, ^3J = 7.1 Hz, 4 H), 2.61 (t, ^3J = 7.4 Hz, 2 H, CH_2), 7.25 (d, ^2J = 8.2 Hz, 2 H, Ar–H), 7.35 (t, ^2J = 5.6 Hz, 2 H, Ar–H), 7.85 (d, ^2J = 7.5 Hz, 2 H, Ar–H), 7.93 (d, 2 H, Ar–H), 8.67 (d, ^2J = 7.5 Hz, 2 H, Ar–H), 8.71 (d, 2 H, Ar–H), 8.73 (d, 2 H, Ar–H). ^{13}**C-NMR** (75 MHz, $CDCl_3$):

δ (ppm) = 171.1,155.1, 154.9, 148.1, 136.4, 135.8, 134.9, 127.4, 122.9, 121.1, 120.4, 117.7, 64.30, 64.18, 33.4, 30.9, 29.9, 28.62, 28.60, 28.58, 28.45, 28.3, 28.1, 27.9, 27.8, 27.7, 27.32, 27.3, 21.7, 18.18, 13.11. **Mass:** ES-MS (DCM–MeOH+10mM NH_4OAc), m/z (%) = 681.4 (100) [MH^+].

Pentacosa-10,12-diynoic acid bis-pyridin-2-ylmethyl-amide(8). Compound **4** (125 mg, 0.63 mmol) was dissolved in dry dichloromethane under N_2 atmosphere, to this solution compound **1** (246.5 mg, 0.63 mmol), DMAP (76.8 mg, 0.63 mmol) and DCC (144 mg, 0.7 m mol) was added. This reaction mixture was stirred at room temperature for 24 h, and then the reaction mixture was filtered and the clear solution was evaporated to dryness. The crude product was purified by silica column chromatography using dichloromethane and petroleum ether as the eluent. The desired compound **8** was collected as colourless solid in the second fraction. **Yield**: 230 mg (68%). **MP**: 95–100 °C. ^{1}**H-NMR** (300 MHz, $CDCl_3$): δ (ppm) = 0.81 (t, ^3J = 7.1 Hz, 3 H, CH_3), 1.40 (s, 26 H, CH_2), 1.51 (t, ^3J = 7.5 Hz, 4 H, CH_2), 1.66 (t, 2 H, CH_2), 2.21 (t, ^3J = 7.1 Hz, 4 H), 2.42 (t, 2 H, –CH_2), 4.73 (s, 2 H, CH_2), 4.79 (s, 2H, CH_2), 7.15–7.35 (m, 4 H, Py), 7.65 (t, 2 H, Py), 8.56–8.48 (dd, 2 H, Py). ^{13}**C-NMR** (75 MHz, $CDCl_3$): δ (ppm) = 174.15, 157.5, 156.9, 149.8, 148.8, 137.0, 122.6, 120.6, 65.2, 65.23, 54.02, 49.6, 33.1, 31.9, 29.6, 29.4, 29.36, 29.28, 29.23, 29.10, 28.94, 28.87, 28.78, 28.36, 28.31, 25.16, 22.7, 19.2, 14.1. **MS:** ES-MS (DCM–MeOH+10mM NH_4OAc), m/z(%) = 556.3(100) [MH^+]. **HRMS** (EI-MS): Calcd for $C_{37}H_{53}N_3O$ [M+]: 555.4189. Found 555.4186.

Pentacosa-10,12-diynoic acid di-pyridin-2-yl-amide (9). Compound **9** was prepared by following the same method as above. 2, 2′-Dipyridyl amine, **5** (100 mg, 0.58 mmol) was dissolved in dry dichloromethane under N_2 atmosphere, to this solution compound **1** (224 mg, 0.60 mmol), DMAP (73 mg. 0.60 mmol) and DCC (123 mg, 0.60 m mol) were added. This reaction mixture was stirred at room temperature for 32 h, and then the reaction mixture was filtered and the clear solution was evaporated to dryness. The crude product was purified by silica column chromatography using dichloromethane and petroleum ether as the eluent. The desired product was collected as the second fraction in the form of colourless solid. **Yield**: 250 mg (72%). ^{1}**H-NMR** (300 MHz, $CDCl_3$): δ (ppm) = 0.87 (t, ^3J = 6.8 Hz, 3 H, CH_3), 1.25–1.33 (m, 26 H, CH_2), 1.51 (m, 24 H, CH_2), 1.69 (t, 2 H, CH_2), 2.23 (t, ^3J = 6.8 Hz, 4 H), 2.40 (d, 2 H, J = 7.8 Hz, CH_2), 6.87 (t, 2H, Ar–H), 7.62 (m, 4 H, Ar–H), 8.21 (t, 2 H, Ar–H). ^{13}**C-NMR** (75 MHz, $CDCl_3$): δ (ppm) = 173.4, 154.1, 148.0, 147.1, 138.1, 116.2, 112.1, 106.5, 77.6, 77.5, 65.3, 39.1, 29.7, 29.64, 29.62, 29.49, 29.36, 29.32, 29.23, 29.19, 28.96, 28.87, 28.82, 28.36, 25.38, 22.71, 19.21, 14.14. **MS:** ES-MS (DCM–MeOH+10mM NH_4OAc), m/z(%) = 528.6(50) [MH^+].

Pentacosa-10,12-diynoic acid (2-diethylamino-ethyl)-amide (10). Compound **6** (50 mg, 0.43 mmol) was dissolved in dry THF under N_2 atmosphere, to this solution compound **2** (167.5 mg, 0.45 mmol), dissolved in THF and dry Et_3N was added. This reaction mixture was stirred at 0° C for 4 h and overnight at room temperature. Then the reaction mixture was filtered and the clear solution was evaporated to dryness. The crude product was purified by silica column chromatography using dichloromethane and methanol as the eluent. The desired product was collected as the second fraction in the form of colourless solid. **Yield**: 145 mg (68%). **MP**: 82–85 °C, ^{1}**H-NMR** (300 MHz, $CDCl_3$): δ (ppm) = 0.75 (t, ^3J = 7.1 Hz, 3 H, CH_3), 1.08 (t, 6 H, CH_3), 1.21 (m, 26 H, CH_2), 1.38 (t, ^3J = 7.5 Hz, 4 H, CH_2), 1.49 (t, 2 H, CH_2), 1.90 (m, 2 H, CH_2), 2.11 (m, 6 H, CH_2), 2.25 (t, ^3J = 7.1 Hz, 2 H), 2.72–2.89 (m, 6 H, CH_2), 3.37 (t, 3 H, –CH_2), 5.48 (s, br, 1 H, NH). ^{13}**C-NMR** (75 MHz, $CDCl_3$): δ (ppm): 173.98, 77.5, 77.4, 65.2, 65.20, 51.9, 49.38, 47.4, 47.3, 36.4, 35.5, 31.9, 30.7, 29.6, 29.6, 29.55, 29.52, 29.4, 29.3, 29.2, 29.13, 29.05, 28.9, 28.8, 28.7, 28.3, 28.3, 25.6, 22.6, 19.1, 19.12, 17.6, 14.1. **MS:** ESI-MS (dichloromethane–MeOH+10mM NH_4OAc), m/z (%) = 473.3 (100). **HRMS** (EI-MS): Calcd. for $C_{31}H_{56}N_2O$ [M+]: 472.4393. Found 472.4386.

Pentacosa-10,12-diynoic acid butylamide (11). Butyl amine **6a** (73 mg, 1 mmol) was dissolved in dry THF under N_2 atmosphere, to this solution compound **2** (393 mg, 1 mmol), dissolved in dry THF and dry Et_3N (0.2 ml) was added. This reaction mixture was stirred at 0° C for 4 h and 16 h at room temperature. Then the reaction mixture was filtered and the clear solution was evaporated to dryness, the crude product was purified by silica column chromatography using dichloromethane and petroleum ether as the eluent. The desired product was collected as the second fraction in the form of colourless solid. **Yield**: 400 mg (81%). ^{1}**H-NMR** (300 MHz, $CDCl_3$): δ (ppm) = 0.84 (t, ^3J = 7.0 Hz, 3 H, CH_3), 0.89 (t, 3 H, CH_3), 1.15–1.32 (m, 28 H, CH_2), 1.45 (m, 6 H, CH_2), 1.55 (t, ^3J = 7.2 Hz, 2 H, CH_2), 2.09 (t, ^3J = 7.6 Hz, 2 H), 2.20 (m, 4 H, CH_2), 3.20 (t, 2 H, CH_2), 5.42 (br, s, 1 H, NH). ^{13}**C-NMR** (75 MHz, $CDCl_3$): δ (ppm) = 173.02, 77.61, 65.28, 65.22, 39.19, 36.90, 31.93, 31.77, 30.32, 29.64, 29.62, 29.66, 29.49, 29.36, 29.23, 29.17, 29.11, 28.92, 28.87, 28.75, 28.36, 28.29, 25.79, 22.70, 20.09, 19.22, 19.19, 14.14.

Acknowledgements

We thank the Deutsche Forschungsgemeinschaft for support of this work. D.A.J. thanks the Alexander von Humboldt foundation for a fellowship

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