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Reversible metal-directed assembly of clusters of vesicles

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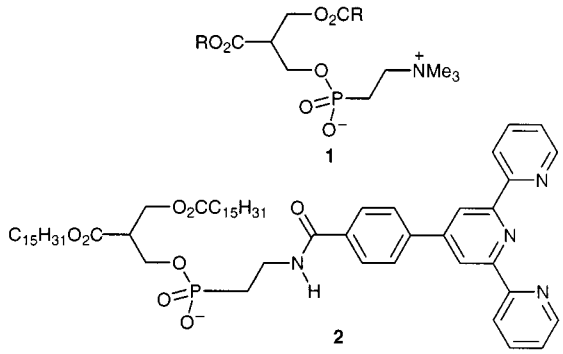
A terpy-functionalised phospholipid has been incorporated into lecithin vesicles and iron(II)-directed aggregation processes have been studied.

Self-assembly is a powerful methodology in supramolecular chemistry that allows the efficient formation of complex multidimensional structures from simple building blocks.[1] Much effort has been devoted to understanding the basic physical chemistry of self-assembled monolayers (SAMs), vesicles and micelles.[2] We have recently commenced a programme in which structural motifs with known self-assembly or molecular recognition paradigms are functionalised with metal-binding domains[3–5] and have described the formation and metal-initiated aggregation of SAMs bearing pendant 2,2′:6′,2″-terpyridine (terpy) motifs.[5] Here, we show that the introduction of a small amount of a terpy-functionalised phospholipid into 'normal' phospholipid vesicles leads to metal-initiated clustering and the formation of aggregates.

Hydrogenated L_α-lecithin **1** (a mixture of phospholipids with 31% R = $C_{16}H_{33}$, 59.75% R = $C_{18}H_{37}$, 5.71% R = $C_{20}H_{41}$ and 3.07% R = $C_{22}H_{45}$, average molecular mass 557 Da) was used as the host. A vesicle stock solution at a L_α-lecithin concentration of 10 mg cm^{-3} (1.8×10^{-2} M) was prepared;† the average vesicle mass was 3×10^7 g mol^{-1} corresponding to 54 000 molecules per vesicle and they have a mean hydrodynamic radius R_h of 54 nm and radius of gyration R_G of 51 nm as determined by dynamic and static light scattering.[6]‡ This gives $\rho = R_G/R_h = 0.944$ which is close to the theoretical value of $\rho = 1.0$ for hollow spheres.[7] The terpy-functionalised phospholipid **2** was prepared as previously described[5] and a 3 wt% amount added to the stock solution of the lecithin to give a total concentration of **2** of 3.29×10^{-4} M. The experimentally measured R_h and R_G were unchanged by the addition of the functionalised compound **2**, indicating no fundamental changes in the solution behaviour of **1** at this level of doping with **2**. The doping corresponds to an average of 970 molecules of **2** per vesicle. Freeze fracture replication transmission electron microscopy[8] revealed the presence of dispersed vesicles [Fig. 1(a)].



The free ligand **2** forms a typical purple iron(II) complex $[Fe(\mathbf{2})_2]^{2+}$ with a characteristic MLCT absorption maximum at 576 nm. Assuming that K_1 and K_2 for the formation of ML and ML_2 complexes are similar to those for terpy itself,[9] even with large excesses of iron, the 1:1 species is not of importance at *ca.* 0.1 mM ligand concentrations. The addition of aqueous $FeSO_4 \cdot 7H_2O$ to the modified vesicle solution resulted in a purple colour (λ_{max} 577–584 nm), indicating that the terpy is still available for coordination and that an $\{ML_2\}$ species is formed. Titration of aqueous Fe^{2+} into the stock solution reached a maximum absorbance at 582 nm at an M:**2** ratio of 0.7:1, compatible with the speciation calculations above and strongly suggesting that all of the terpy groups are on the *outer* surface of the vesicle.

The results of dynamic light scattering measurements as the iron(II) salt as titrated into the modified vesicle solution are presented in Fig. 2. The measured R_h increases smoothly past the 1:2 Fe^{2+}:**2** equivalence point until an Fe^{2+}:**2** ratio of *ca.* 4.5:1 is reached. After this point a rapid increase in the measured hydrodynamic radius occurs followed by precipitation of a violet solid when $[Fe^{2+}]$ reaches 1.76 mM. A control experiment with the stock solution in the absence of **2** showed

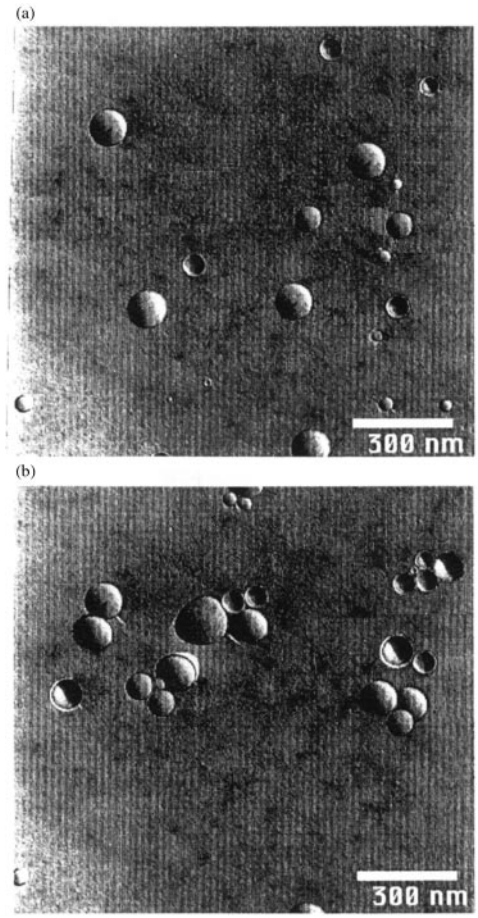


Fig. 1 (a) Freeze fracture electron micrograph of vesicles obtained with lecithin **1** (1.8×10^{-2} M) and **2** (3.29×10^{-4} M). No clustered vesicles are observed. (b) Freeze fracture electron micrograph of an identical vesicle solution after treatment with aqueous iron(II) sulfate to give an Fe^{2+}:**2** ratio of 4.5:1.

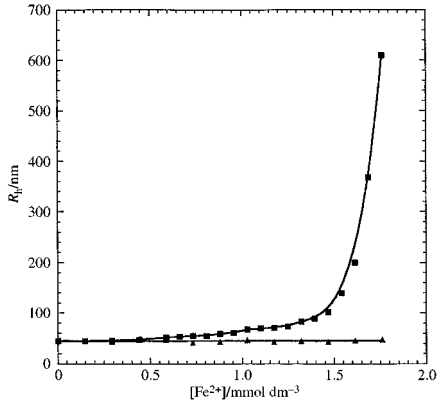
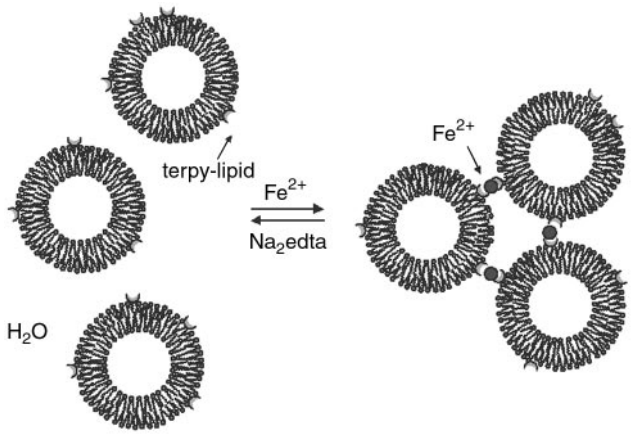


Fig. 2 Measured hydrodynamic radius of vesicles as a function of aqueous iron sulfate concentration. The data presented refer to the hybrid **1** + **2** vesicles (■) and to a control experiment with **1** alone (▲).

that the addition of the iron salt had no effect on the parent vesicles (Fig. 2). The aggregation process proved to be reversible, and the addition of a 100-fold molar excess of $Na_2(H_2edta)$ at 40 °C resulted in the discharge of the violet colour and restoration of the light scattering characteristics of the vesicle solution. At an Fe^{2+}:**2** ratio of 4.5:1, light scattering gave R_G = 108 nm and R_h = 106 nm consistent with an aggregation of the vesicles into clusters (see later). Metal-initiated transformation into giant vesicles can be rejected on the basis of the reversibility of the process upon addition of edta.

We propose that an aggregation process is occurring (Scheme 1) in which the surface terpy ligands are coordinating to the iron(II) centres. The low dilution of **2** within individual vesicles means that inter-vesicle coordination is favoured and each



Scheme 1 Proposed iron(II) induced aggregation process of modified vesicles.

coordination event will clip two vesicles together. The high Fe^{2+}:**2** ratios needed before precipitation occurs represent the statistical factors involved in bringing two ligands together. The dimensions indicate an average aggregation state of 3.1 ± 1.1 vesicles in the clusters at a ratio of Fe^{2+}:**2** of 4.5:1 and an increase in size as the amount of iron is increased. It has previously been reported[10] that biotin-functionalised vesicles may be aggregated into clusters through interaction with streptavidin, but the approach we present here utilises a remarkably simple metal–ligand recognition process for the aggregation.

Direct evidence for the aggregation comes from cryomicroscopy. Fig. 1(b) shows a freeze fracture electron micrograph of the material at a ratio of Fe^{2+}:**2** of 4.5:1. At this concentration, microscopy reveals that only a very few single vesicles are present, the majority being aggregated into clusters.

In conclusion, we have shown that iron(II) salts may be used to assemble modified vesicles into clusters in a reversible process. We are currently investigating the extension of the chemistry to other metal ions with the aim of introducing specific redox, magnetic or photophysical properties into the aggregates.

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Notes and references

† **1** was deposited on the wall of a glass vessel by evaporation of a 1.8 M $CHCl_3$ solution and then hydrated in doubly distilled water to give a multilamellar liposome dispersion. This was repeatedly frozen in liquid nitrogen and thawed, followed by repeated extrusion through Nanopore filters of 100 nm pore size to form unilamellar vesicles.

‡ The results of the static and dynamic light scattering were obtained by extrapolation to zero concentration of vesicles by dilution of the stock solution with doubly distilled water. This indicates that the vesicles and the vesicle clusters are stable under these conditions of dilution.

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