Communications to the Editor

Self-Assembled Columns of Stacked Lipid Bilayers Mediated by Metal Ion Recognition

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Molecular self-assembly can generate elaborate two- and three-dimensional supramolecular structures through the attractive and repulsive interactions between molecular components and their environment.[1] Synthetic self-assembling systems are currently limited to the one-step creation of supramolecular structures from molecular components, unlike biological systems that can organize supramolecular assemblies into higher ordered structures to create tissue and functional materials of specific dimensions.[2] Herein, we report the first observation of a synthetically produced higher ordered self-assembled structure composed of lipid bilayers having self-limiting dimensions mediated by chemical recognition at the membrane surface. Transmission electron microscopy (TEM) images of the self-assembled material reveal a columnar structure of stacked lipid bilayers uniform in width with lengths spanning dozens of bilayer thickness.

Previous work found that liposomes composed of a pyrene-labeled, synthetic receptor lipid mixed into a distearylphosphatidylcholine (DSPC) matrix performed as highly selective optical sensors for heavy metal ions.[3−5] In the absence of metal ions, the liquid-phase receptor lipid separates from the solid-phase DSPC matrix, producing fluorescence spectra with large excimer emission ($\lambda_{max} = 470$ nm) and relatively small monomer emission ($\lambda_{max} = 375$ nm). Addition of di- or trivalent metal ions causes an inversion of the fluorescence emission peaks (excimer emission attenuates as the monomer emission intensifies), revealing dispersion of the receptor lipids into the DSPC matrix upon the metal ion recognition. Complete reversibility of the process is possible by removal of the metal ions with EDTA.

Within these studies we noticed that the addition of Cu^{2+} to lipid bilayers composed of 5% PSIDA/DSPC (Figure 1) at a relatively high lipid concentration 0.1 mM caused the solution to become turbid, indicative of vesicle aggregation. The turbidity was, like the metal ion response, reversible upon the addition of EDTA. Turbid solutions were not observed with other receptor lipids functionalized with dithioamide or a crown ether that were selective for Hg^{2+} or Pb^{2+}, respectively, nor with the 5% PSIDA/DSPC liposomes in the presence of other divalent metal ions, such as Mn^{2+} or Ca^{2+}.

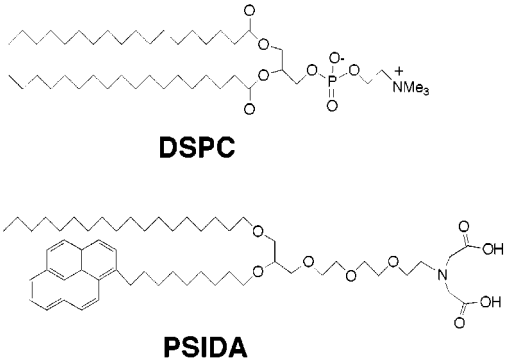

Figure 1. Matrix lipid DSPC and pyrene-labeled lipid PSIDA.

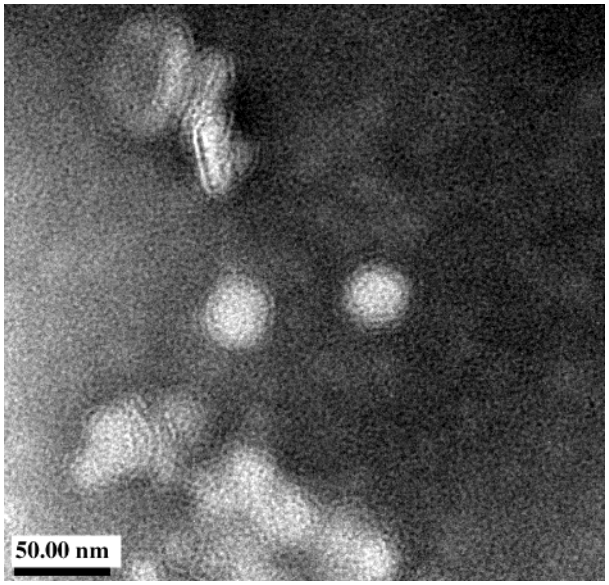

Figure 2. TEM image of 5% PSIDA/DSPC bilayers in MOPS buffered water at pH 7.4 (ammonium molybdate stain).

In an effort to characterize the aggregate structures formed upon metal ion recognition, the bilayers were imaged by electron microscopy before and after addition of Cu^{2+}. The liposomes were prepared as described previously,[5] then negatively stained using a standard TEM preparation protocol.[6] TEM images were taken on a Philips CM-30 operated at 300 kV. Figure 2 is an image of small unilamellar vesicles (SUV) of 5% PSIDA/DSPC bilayers formed in MOPS buffered water (0.02 M MOPS, 0.10 M NaCl) at pH 7.4. Two free-floating liposome structures are observed in the middle of the image. Liposome sizes ranged between 400 and 700 nm in diameter. In addition, various forms of unstructured bilayer aggregates can be seen on the top and bottom of the image, accounting for approximately 50% of the observed features.

Upon addition of Cu^{2+} (1.0 μM) to this solution a remarkable self-assembly of the bilayers into columnar structures results. A representative image is shown in Figure 3, with more images available in the Supporting Information. Approximately 15−20% of the observed bilayer structures on the TEM grid were in the form of these columns, the rest as aggregates and free liposomes. These unique structures have been reproducibly prepared.

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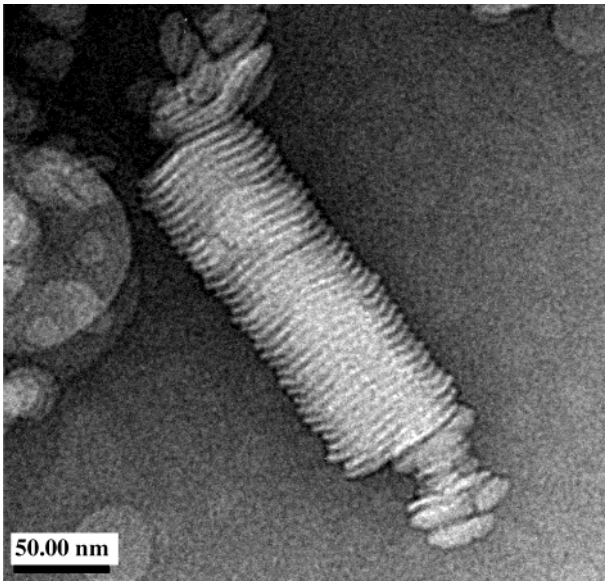

Figure 3. TEM image of a self-assembled, columnar structured lipid bilayer stack that forms after $CuCl_2$ (1.0 μM) addition to liposomes of 5% PSIDA/DSPC.

The bilayer stacks exhibit uniform width throughout most of the structure. Other self-organizing lipid bilayer structures formed through bilayer-DNA[7] or bilayer-actin[8] complexation, metal ion coodination,[9] or biotin-streptavidin molecular recognition[10] do not exhibit such self-limiting dimensions. These columnar structures varied in width from 600 to 900 Å with lengths between several (300 Å) to ~45 (3300 Å) bilayer thickness. The structure appears to be composed of individual lipid bilayers ~40 Å thick with ~30 Å spacing between each layer. It does not appear that these are flattened liposomes for the edges of each bilayer in the stack are discreet with no connectivity with its adjacent neighbor. On one end of each stack a short, poorly organized stack of bilayers exists that is approximately half the diameter of the column (bottom of Figure 3). At the other end, the stacks tend to taper off in size in the final few bilayers, sometimes leading off to another poorly organized stack (top of Figure 3).

The mechanism of formation of these self-assembled structures with uniform width is difficult to imagine considering that the initial liposome sizes are polydisperse. From the fluorescence data, we do know that Cu^{2+} binding to the iminodiacetic acid (IDA) receptor induces a dispersion of PSIDA molecules into the lipid matrix. Coordination of Cu^{2+} to the IDA headgroup leaves open an equivalent coordination site for another IDA to bind. The fluorescence data also indicate that intraliposome 2:1 coordination of IDA to Cu^{2+} does not occur, most probably due to geometric constraints of the two-dimensional surface. Orientation of the Cu^{2+}−IDA complex perpendicular to the surface would, however,

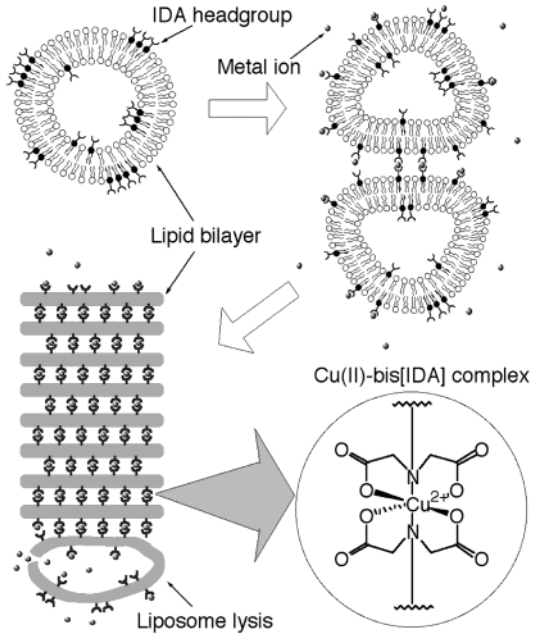

Figure 4. Proposed mechanism of lipid bilayer stack formation. The top left structure represents the initial liposome. Following metal ion addition, liposomes adhere to each other through Cu^{2+}-bis[IDA] complexation (right) causing membrane flattening. Subsequent adhesion and liposome lysis leads to bilayer stacking (bottom left).

allow complexation with an IDA from another liposome. Through this metal ion-coordination-mediated event, numerous adhesion points between the two liposomes would rapidly propagate resulting in a flattening of the opposing bilayers, as illustrated in Figure 4. The high affinity of Cu^{2+} to IDA should facilitate the process, whereas ions with low affinity (eg. Mn^{2+}, Ca^{2+}) may not provide sufficient adhesion. The dispersion of receptors due to metal ion binding should further aid in the uniform adhesion between the bilayer surfaces. At some point the liposome ruptures as a result of deformation from the adhesion process, allowing Cu^{2+} to reach receptors in the liposome's interior. Although we have no confirming images at this time, the bilayers are most likely in the shape of a disk, as one might think of a flattened sphere. The first one or two bilayers formed may serve as a template for successive stacking of bilayers, regulating the width through the adhesion points between bilayers. The self-organizing nature of lipid assemblies may allow the bilayers to restructure, either recruiting or displacing lipids from adjacent bilayers to form a contiguous bilayer that matches the template size.

Investigations into the mechanism of formation of these bilayer stacks are currently underway. Such structurally defined, asymmetric materials may have potential use as scaffolding in nanoarchitecture or as structures with unique chemical or optical function.

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Supporting Information Available: Additional TEM images of lipid bilayer stacks (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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