Lipid Bilayers

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Amphiphilic Nanoparticles Control the Growth and Stability of Lipid Bilayers with Open Edges

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Abstract: *Molecular amphiphiles self-assemble in polar media to form ordered structures such as micelles and vesicles essential to a broad range of industrial and biological processes. Some of these architectures such as bilayer sheets, helical ribbons, and hollow tubules are potentially useful but inherently unstable owing to the presence of open edges that expose the hydrophobic bilayer core. Here, we describe a strategy to stabilize open bilayer structures using amphiphilic nanoparticle surfactants that present mixtures of hydrophilic and hydrophobic ligands on their surface. We observe that these particles bind selectively to the open edge of bilayer membranes to stabilize otherwise transient amphiphile assemblies. We show how such particles can precisely control the size of lipid tubules, how they can inhibit the formation of undesirable assemblies such as gallstone precursors, and how they can stabilize free-floating lipid microdiscs.*

Molecular amphiphiles are prototypical building blocks of many self-assembled structures such as vesicles and membranes that provide stable compartments essential for drug delivery,[1] food products, cosmetics, and even life itself.[2] The architectures formed by these components are typically limited to closed structures, in which the hydrophobic domains are carefully concealed from their polar surroundings. En route to these thermodynamically favored architectures, however, amphiphiles can form metastable structures such as bilayer sheets, helical ribbons,[3] or hollow tubules[4] that present open edges exposing the hydrophobic core of the bilayer. The ability to stabilize such structures is desirable in several contexts from the study and application of membrane proteins[5] to the templated synthesis of inorganic nanomaterials.[6] While certain detergents and proteins have been shown to stabilize open lipid structures such as bicelles[7] and nanodiscs,[8] there exists no general strategy for controlling the growth and stability of lipid assemblies with open edges. Nanoparticles (NPs) with appropriate surface chemistries are attractive candidates for stabilizing such structures, as their size can be made commensurate with the bilayer thickness, thereby facilitating multivalent interactions with the bilayer edge. Surface-active nanoparticles are known to adsorb strongly at oil–water interfaces[9] and to various types of bilayer membranes.[10] In particular, amphiphilic nanoparticles with hydrophilic and hydrophobic domains on their surface can form stable dispersions in water[11] and interact strongly with the hydrophobic core of bilayer membranes.[11a,12]

Here, we show that amphiphilic NPs can bind selectively to the open edge of bilayer membranes to stabilize otherwise transient structures and inhibit their further growth. The method we describe is based on gold NPs (6.2 ± 0.8 nm in diameter) functionalized with mixed monolayers of hydrophilic (TMA) and hydrophobic (ODT or DDT) ligands in a ratio of 77:23 unless otherwise stated (Figure 1a; see Supporting Information for experimental details).[11] As shown below, addition of these particles to solutions containing open bilayer structures resulted in the selective adsorption of particles at the hydrophobic edge of the membrane. The resulting NP capped bilayers were stable against further growth and remained unaggregated in solution for several weeks. To demonstrate the generality of this mechanism, we investigated the effects of amphiphilic NPs on three different bilayer-forming materials: 1) hollow tubules of $DC_{8,9}PC$ lipid,[4] 2) helical ribbons of cholesterol,[3] and 3) sheared vesicles of DPPC lipid.[13] Each material is used to highlight a different opportunity for applying these NP surfactants 1) to precisely control the dimensions of lipid nanostructures, 2) to inhibit the growth of undesirable assemblies such as cholesterol gallstones, and 3) to stabilize lipid microdiscs used in the study of membrane proteins. Taken together, these results suggest that amphiphilic NPs can act as versatile supramolecular surfactants, which bind to select surfaces and control their growth.[14]

The first material we considered is one of several amphiphiles[4,15] that self-assemble from solution to form hollow tube-like architectures (Figure 1b). These micron-scale tubules are useful as containers for controlled release,[16] as templates for material synthesis,[6] and as colorimetric biosensors.[17] Although several of these applications depend critically on the tubule dimensions, it remains challenging to prepare tubules of a desired length and to prevent further growth from their ends. In a typical experiment, lipid tubules were prepared by quenching a solution of $DC_{8,9}PC$ vesicles in liquid nitrogen for 1 min and then annealing at room temperature for hours to days. The rapid cooling induced a phase transition[18] in the bilayer structure that disrupted the vesicles and induced the formation of hollow tubules ca. 500 nm in diameter with an average length of 20.0 ± 0.8 μm as evidenced

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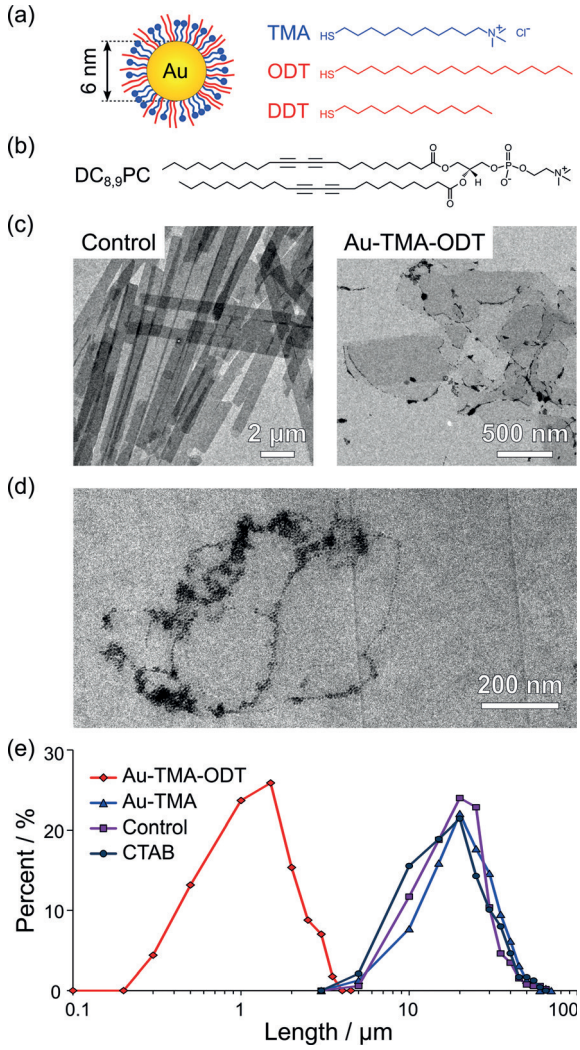

Figure 1. Amphiphilic NPs control the growth of lipid tubules. a) Schematic illustration of an AuNP functionalized with hydrophilic (TMA) and hydrophobic (ODT or DDT) ligands. b) The lipid $DC_{8,9}PC$ is known to self-assemble in solution to form hollow tubules. c) Conventional TEM images showing large tubules grown in the absence of the particles (left) and small bilayer sheets stabilized by amphiphilic AuNPs (right). d) Cryo-TEM image of a folded bilayer sheet stabilized by amphiphilic AuNPs adsorbed along its edge. e) Length distributions of tubules assembled in the presence of amphiphilic Au-TMA-ODT NPs, hydrophilic Au-TMA NPs, or the molecular surfactant CTAB as compared to control experiments with no additives.

by transmission electron microscopy (TEM; Figure 1c) and optical microscopy (OM; Figure S3).

By contrast, when Au-TMA-ODT NPs were added to the lipid solution prior to quenching in liquid nitrogen (in 3:2 ratio of Au atoms to lipid), no tubules were observed even after several weeks of annealing at room temperature. Instead, the amphiphilic NPs stabilized the formation of small bilayer sheets (1.3 ± 0.1 μm in diameter) as revealed by conventional TEM (Figure 1c) and by cryo-TEM (Figure 1d). The electron micrographs show that the amphiphilic NPs adsorbed selectively along the open edges of the lipid bilayer.

Both hydrophilic TMA and hydrophobic ODT ligands were necessary to observe association between NPs and lipid bilayers. Hydrophobic Au-ODT NPs precipitated rapidly upon addition to the lipid solution (Figure S4). By contrast, hydrophilic Au-TMA NPs were readily dispersed without aggregation but showed no specific association with the bilayer structure (Figure S5). In the presence of Au-TMA NPs, lipids assembled to form long tubules (Figure S3) identical to those observed in the absence of the particles (Figure 1e). These experiments suggest that hydrophobic ODT ligands are needed to facilitate attractive hydrophobic interactions between the NPs and the bilayer edge. At the same time, hydrophilic TMA ligands contribute both to the solubility of the NPs in solution and to the stability of the NP-capped bilayer sheets.

Based on these observations, we propose that amphiphilic NPs adsorb strongly at the bilayer edge via hydrophobic interactions mediated by ODT ligands on the particles' surface. Previous reports have suggested that mixtures of thiolate ligands presenting hydrophobic and hydrophilic surface groups can rearrange to form hydrophobic "patches" in response to chemical heterogeneity in the particles' environment (e.g., at liquid interfaces,[19] within micellar clusters,[11b,20] or in bilayer membranes[11a,12b,c]). We hypothesize that amphiphilic NPs interact with the bilayer edge through one such hydrophobic domain enriched with ODT ligands. As a rough approximation, the strength of this hydrophobic interaction is of the order $4\pi a^2 f\gamma \approx 60\, k_B T$ where $a = 3$ nm is the NP radius, $f = 0.23$ is the ODT surface fraction, $\gamma = 10\ \mathrm{mN\,m^{-2}}$ is the interfacial tension, and $k_B T$ is the thermal energy at room temperature. These strong attractive interactions are consistent with cryo-TEM images, which show that nearly all NPs in solution were adsorbed at the bilayer edge. Once attached to the bilayer, the amphiphilic NPs likely form a physical barrier that inhibits both the merging of two bilayer sheets as well as the addition of individual lipids.

Consistent with the proposed mechanism, the nanoscopic size of the amphiphilic particles was critical to arresting tubule growth, as it allowed for multivalent interactions with the bilayer edge. By contrast, the addition of a chemically similar, molecular amphiphile—the cationic surfactant, cetyltrimethyl-ammonium bromide (CTAB)—to the lipid solution had no effect on tubule growth (Figure 1e). Additionally, amphiphilic NPs of identical size but with fewer hydrophobic ligands (90:10 TMA to ODT) were also ineffective at inhibiting tubule growth (Figure S7). This observation can be rationalized qualitatively by considering the maximum size of the putative hydrophobic patch as compared to the thickness of the lipid bilayer (here, 6.5 nm[21] or 4.8 nm for the hydrophobic bilayer core). Decreasing the ODT fraction from 23% to 10% reduces the patch size from 5.2 nm to 3.7 nm, such that it can no longer span the entire thickness of the hydrophobic bilayer edge (see Supporting Information for details). As a result, the more hydrophilic particles likely form a weaker and more permeable barrier, which is incapable of preventing further tubule growth.

The ability of amphiphilic NPs to rapidly arrest tubule growth can be exploited to control the length of the tubules by

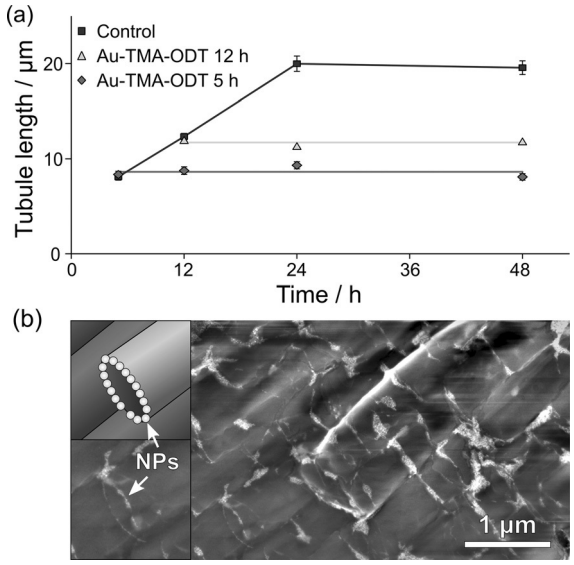

Figure 2. NPs control tubule length. a) Desired tubule length can be achieved by varying the time at which amphiphilic NPs are injected to the lipid solution (injection after 5 h, diamonds; injection after 12 h, triangles). b) SEM image showing tubules stabilized by amphiphilic AuNPs adsorbed at their ends. The insets show a schematic illustration and a magnified image of the NP-capped tubule ends.

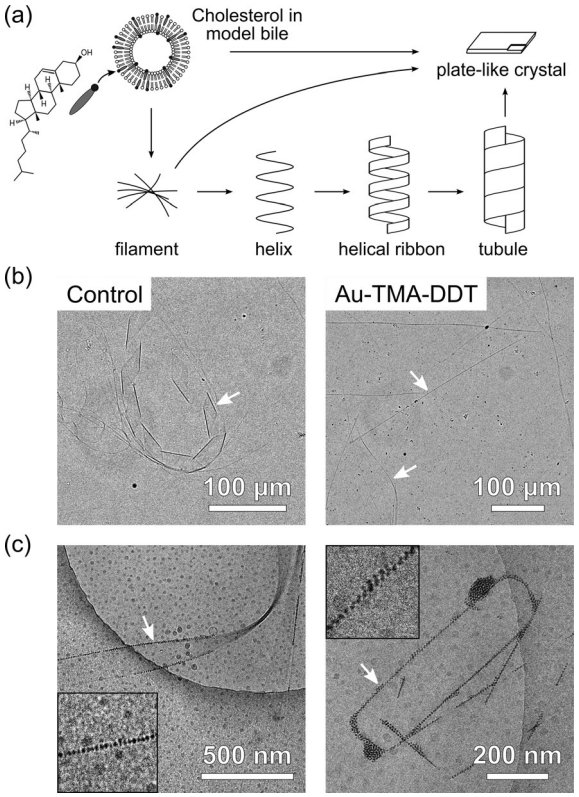

Figure 3. Amphiphilic NPs inhibit cholesterol crystallization from model bile. a) Structural evolution of cholesterol crystallization from model bile. b) OM images showing helical ribbons of cholesterol grown in the absence of the particles (left) and thin filaments stabilized by amphiphilic AuNPs (right). c) Cryo-TEM images of cholesterol filaments stabilized by Au-TMA-DDT NPs adsorbed at the edge of the filaments.

varying the time at which NPs are added (Figure 2). In the absence of the particles, the average tubule length increased steadily to ca. 20 µm during the first 24 h of annealing and remained so thereafter due to the depletion of free lipid from solution. When, however, amphiphilic NPs were added to the lipid solution after only 5 h of annealing, further growth of the tubules ceased, and the average tubule length remained constant at 8.6 ± 0.5 µm for several days (Figure 2a). Scanning electron microscope (SEM) images revealed that the NPs adsorbed preferentially to the growing tubule edges thereby arresting their further growth (Figure 2b). These observations indicate that tubules of desired length can be prepared by simply injecting NP amphiphiles into the lipid solution at a particular time during their growth.

Importantly, the above mechanism for controlling the growth and stability of bilayer structures should be generally applicable to other materials that form metastable intermediates with open bilayer edges. To test this hypothesis, we investigated the effects of amphiphilic NPs on the crystallization of cholesterol from a model bile solution, which proceeds through several structural intermediates—filaments, helical ribbons, and tubules—en route to the formation of bulk crystals[3] (Figure 3a). These structures are relevant to the biological formation of gallstones,[22] and the ability to arrest their growth could suggest possible strategies for the prevention of gallstone-associated ailments such as ascending cholangitis or pancreatitis. In a typical experiment, a model bile solution containing cholesterol, lecithin, and bile salts in water was incubated over a 2-week period. Initially, cholesterol crystals nucleated and grew to form long filamentous ribbons, which evolved in time into helical ribbons, closed tubules, and ultimately plate-like crystals. After 9 days of incubation, mixtures of these various structures could be observed to coexist by OM (Figure 3b).

By contrast, when AuNPs functionalized with hydrophilic TMA and hydrophobic DDT ligands were added to the supersaturated bile solution after 1 day of incubation (in 2:1 ratio of Au atoms to cholesterol), they adsorbed at the growing edge of the cholesterol filaments thereby inhibiting the formation of ribbons and tubules. After 9 days of incubation, we observed a mixture of thin filaments and few plate-like crystals with none of the helical ribbons or tubules seen in control experiments without the NPs (Figure 3b). Cryo-TEM revealed that amphiphilic NPs adsorbed selectively along the edges of the cholesterol filaments (Figure 3c, Figure S10), which are known to have a bilayer structure with hydrophilic (hydroxy-rich) faces and hydrophobic edges.[23]

Although amphiphilic NPs were effective in arresting the growth of cholesterol filaments, they were unable to completely inhibit the formation of plate-like crystals, which can form directly via nucleation and growth (bypassing the kinetically controlled ribbon and tubule structures). Nevertheless, crystals grown in the presence of the particles exhibit unusual morphologies that reflect the retarding effects of NP adsorption on crystal growth (Figure S11). With further optimization, similar strategies could more effectively inhibit this and other undesirable crystallization processes.

As a third and final demonstration of the stabilizing effects of amphiphilic NPs on open bilayer membranes, we considered the transient lipid sheets formed by shearing dispersions of DPPC vesicles, commonly used in model cell membranes[13] (Figure 4a). In the absence of the NPs,

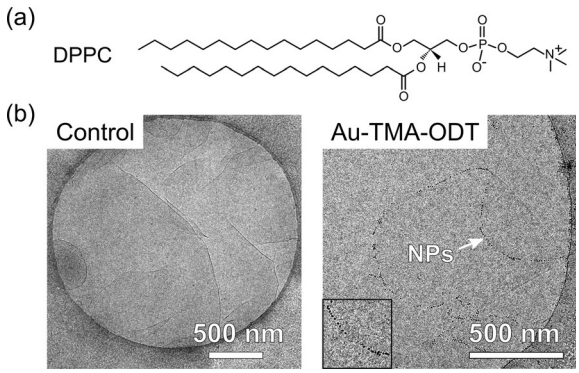

Figure 4. Amphiphilic NPs stabilize DPPC sheets. a) The lipid DPPC self-assembles in solution to form spherical vesicles. b) Cryo-TEM images showing large bilayer sheets formed by shearing a solution of DPPC vesicles (left) and small bilayer sheets stabilized by amphiphilic AuNPs (right). The inset shows a magnified image of the bilayer edge decorated with AuNPs.

vigorous stirring of solutions containing spherical vesicles ca. 50 nm in diameter caused the vesicles to rupture and fuse to form large bilayer sheets up to several microns in size as observed by cryo-TEM (Figure 4b). By contrast, when Au-TMA-ODT NPs were added to the vesicle solution prior to stirring (in 1:4 ratio of Au atoms to lipid), we observed that the shear-induced membrane sheets were much smaller (ca. 100 nm) than those prepared in the particles' absence (Figure 4b; see also Figure S12). Consistent with our previous observations, cryo-TEM images show that the NPs adsorbed along the open edge of the bilayer membranes (Figure 4b, Figure S13), which remained stable in solution even after stirring ceased. The final size of the NP-capped bilayers was comparable to that of the initial vesicles, which suggests that the particles adsorbed to the shear-ruptured vesicles before they were able to grow and merge to form larger sheets. These lipid sheets—or "microdiscs"—are potentially useful in the study and application of membrane proteins,[5] as they can offer larger sizes than existing nanodiscs[8] and greater stability than detergent-stabilized bicelles.[7]

In sum, we have shown that amphiphilic NPs can act as versatile supramolecular surfactants[14] that bind strongly and selectively to open bilayer membranes to stabilize amphiphile assemblies. This general strategy is not unlike that of antifreeze proteins,[24] which adsorb to selective crystal facets to kinetically arrest the growth of undesired ice within certain organisms. There, like here, the strength and selectivity of the interactions derive from the coordinated action of many weaker bonds between chemically heterogeneous, nanoscale surfaces.[25] Future applications of these NP surfactants could further capitalize on the unique material properties such as plasmonic or magnetic responses that derive from the particles' inorganic cores.

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