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Controlled Self-Assembly of Filled Micelles on Nanotubes

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Supporting Information

ABSTRACT: We have used coarse-grained molecular dynamics simulations to show that hydrated lipid micelles of preferred sizes and amounts of filling with hydrophobic molecules can be self-assembled on the surfaces of carbon nanotubes. We simulated micelle formation on a hydrated (40,0) carbon nanotube with an open end that was covered with amphiphilic double-headed $CH_3(CH_2)_{14}CH(((CH_2OCH_2CH_2)_2(CH_2COCH_2))_2H)_2$ or single-headed $CH_3(CH_2)_{14}CH_2((CH_2OCH_2CH_2)_2(CH_2COCH_2))_4H$ lipids and filled with hexadecane molecules. Once the hexadecane molecules inside the nanotube were pressurized and the lipids on its surface were dragged by the water flowing around it, kinetically stable micelles filled with hexadecane molecules were sequentially formed at the nanotube tip. We investigated the stability of the thus-formed kinetically stable filled micelles and compared them with thermodynamically stable filled micelles that were self-assembled in the solution.

In recent years, nanomedicines with unique characteristics have been developed.[1,2] Among the many tested systems, lipid and polymeric micelles[3] and nanoparticles self-assembled from amphiphilic block copolymers[4] have become particularly promising drug nanocarriers. Typically, the micelle carriers have multishell molecular structures with compact hydrophobic cores separated by narrow charged layers from loosely ordered hydrophilic external shells.[5] These chemically distinct layers allow nesting and delivery of different drugs,[6] proteins,[7] and other molecules that are poorly soluble in water.[8,9]

Hydrated amphiphilic molecules, such as phospholipids or surfactants, tend to self-assemble into *thermodynamically stable* micelles above their critical micelle concentrations (CMCs).[10] The micelles can be filled in a stepwise manner with hydrophobic molecules,[5] but their stabilization may be a relatively complex process. Moreover, the average sizes and shapes of these micelles and the amounts of molecular cargo filling them are determined by the character of the monomers and the solutions used in their preparation. In various applications, it might be of interest to prepare *kinetically stable* micelles with relatively long lifetimes that can have controllable sizes and be filled with different types and numbers of molecules.

In this work, we investigated the possibility of controlling the self-assembly of kinetically stable micelles prepared and filled with a molecular cargo at nanoscale material surfaces. We used the fact that hydrated lipids spontaneously form hemimicelles[11] or cylindrical micelles,[12] depending on the type of lipids used,[13−15] on the hydrophobic surfaces of carbon nanotubes (CNTs).[16,17] These premicelles might be dragged on the CNT surfaces,[18−20] filled with molecules at the CNT tips, and sequentially released, in close analogy to microsyringes or nanoscopic jets.[21] The amphiphilic lipids forming the micelles can be adsorbed on the CNTs from the solution, and the molecules filling the micelles could be continuously supported through the CNT interior. In dip-pen nanolithography,[22] various molecules can be analogously deposited on material surfaces by nanoscopic tips.

We studied the self-assembly of filled micelles on CNT surfaces by classical molecular dynamics (MD) simulations. In order to describe these large systems with long and complex dynamics,[23,24] we performed coarse-grained MD (CGMD) simulations[25−28] with the NAMD package[29−31] and the Martini 2.0 force field.[28] We modeled two types of amphiphilic lipids that potentially can form different micelles,[14] having either one $[CH_3(CH_2)_{14}CH_2((CH_2OCH_2CH_2)_2(CH_2COCH_2))_4H]$ or two $[CH_3(CH_2)_{14}CH(((CH_2OCH_2CH_2)_2(CH_2COCH_2))_2H)_2]$ hydrophilic heads and a single hydrophobic tail.

Coarse graining of the lipids was performed through a four-to-one atom-mapping procedure[28] where every four non-hydrogen atoms in the lipids were modeled as a single bead. The CG hydrophobic tails were represented by C_1-type beads [$CH_3(CH_2)_2CH_3$, butane],[28] while the hydrophilic heads were represented by N_a-type (C_3=O, 2-propanone) and N_0-type (C−O−C_2, methoxyethane) beads. We used 2:1 mapping[28] of the carbon atoms in the considered (40,0) CG CNT in order to preserve its hexagonal symmetry. Every two neighboring carbons in the all-atom CNT were modeled as an SC_4-type bead. Similarly, every four water molecules were united into a single P_4-type bead,[28] and the hydrophobic hexadecane molecules filling the CNT were modeled by C_1-type beads.[28]

First, we studied the self-assembly of biheaded lipids, shown in the inset of Figure 1. The large size (two chains) of their polar heads relative to their hydrophobic tails means that these hydrated lipids (alone) prefer to self-assemble into smaller micelles.[14] In our simulations, we fixed the CNT, covered it with 450 lipids, and filled it with 400 hexadecane molecules. The system was hydrated, placed in a cell of dimensions 16 nm × 16 nm × 70 nm with periodic boundary conditions applied, and simulated at T = 350 K in the *NPT* ensemble. We used the Langevin piston method[32] with a damping coefficient of 1 ps^{-1}. In order to prevent artificial freezing of CGMD water at low damping, we used antifreeze water beads (8% of the total normal water beads).[28]

Figure 1 shows the sequential self-assembly of lipid micelles with a controllable amount of hexadecane filling on the (40,0) CNT. Upon deposition on the CNT, these lipids formed random hemimicelles within 20 ns,[16,33,34] as shown in Figure 1a. Next, we

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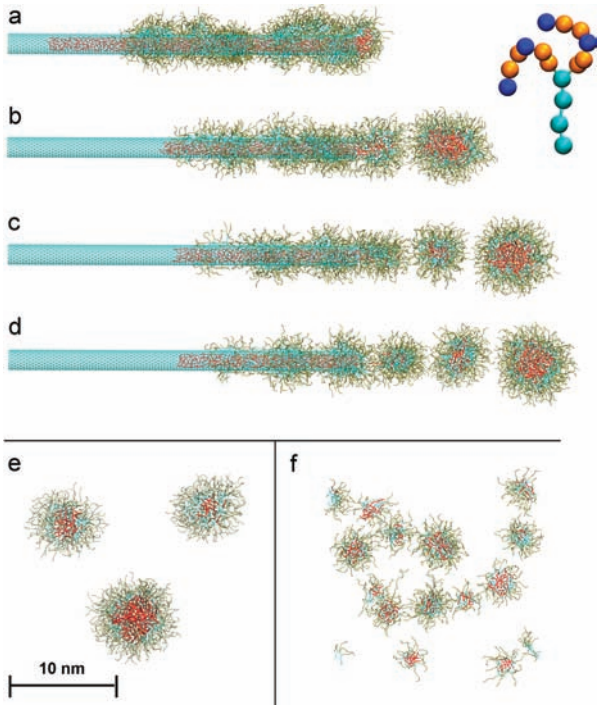

Figure 1. Controlled self-assembly of hydrated double-headed lipid micelles filled with hexadecane. (a) Lipids deposited on the CNT form random hemimicelles after 20 ns. (b–d) Micelles with kinetically controlled sizes and filling are sequentially formed at the CNT tip when the hexadecane molecules in the CNT interior are pressurized and the lipids on its surface are dragged by the water flowing around it. The inset shows a single biheaded lipid molecule. Color scheme: red, CG hexadecane; gray, hydrophilic lipid head; cyan, hydrophobic lipid tail. See the movie in the Supporting Information. (e) Three hexadecane-filled micelles from (d) after 400 ns of equilibration. (f) Thermodynamically driven self-assembly of double-headed lipid monomers and hexadecane in water for the same system as in (d). After 300 ns of equilibration, the sizes of the micelles and their filling are largely random.

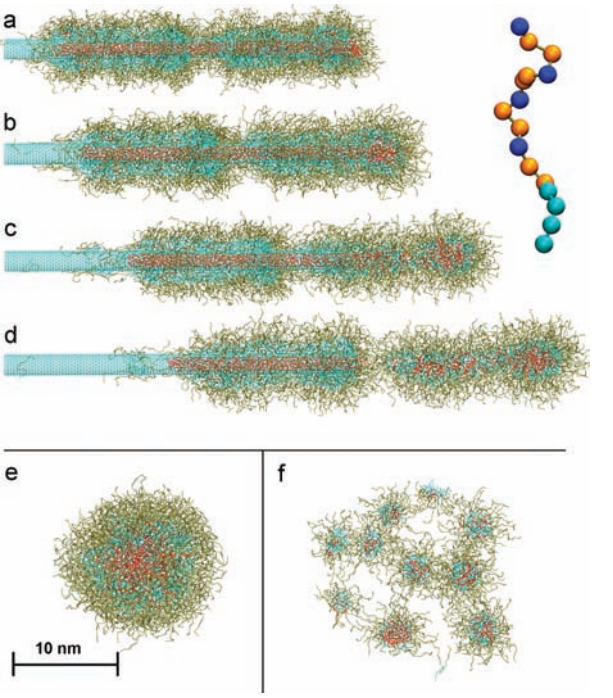

Figure 2. Controlled self-assembly of hydrated single-headed lipid bicelles filled with hexadecane. (a) Lipids deposited on the CNT form random tubular structures after 20 ns. (b–d) Bicelles with kinetically controlled sizes and filling are sequentially formed at the CNT tip when the hexadecane molecules in the CNT interior are pressurized and the lipids on its surface are dragged by the water flowing around it. The inset shows single single-headed lipid molecule. Color scheme: red, CG hexadecane; gray, hydrophilic lipid head; cyan, hydrophobic lipid tail. See the movie in the Supporting Information. (e) Hexadecane-filled bicelle from (d) after 375 ns of equilibration. (f) Thermodynamically driven self-assembly of single-headed lipid monomers and hexadecane in water for the same system as in (d). After 450 ns of equilibration, the sizes of the micelles and their filling are largely random.

applied on each water bead the force f_1 = 2.08 pN oriented toward the CNT right tip. As a result, water flowed with average velocity v_t = 1.5 m/s and dragged the lipids adsorbed on the CNT surface. Hexadecane molecules inside the CNT were also pressurized by application of the force f_2 = 1.39 pN on each of their beads, oriented in the same way as f_1. In experiments, loosely separated CNTs may be fixed and their entrances and exits may be immersed in two separated solutions. The external solution might be pumped to flow around the adsorbed lipids, while the intercalated molecules could be driven by pressure.

Upon application of these forces, the lipid and hexadecane molecules started to move toward the CNT tip, and soon the first hemimicelle covered the CNT tip. Within 4 ns, a micelle of diameter $d \approx 5$ nm containing ≈100 hexadecane molecules formed and detached from the CNT tip, as shown in Figure 1b. When it left, the remaining lipids and hexadecane molecules moved forward, and the next hemimicelle covered the CNT tip in a similar fashion. The second filled micelle had $d \approx 3$ nm and contained ≈40 molecules. The third filled micelle was then created, and so on. The formed micelles had different sizes, since the progression of both sets of molecules did not remain steady as their amounts decreased. As the micelles formed and detached, fewer hexadecane molecules were present inside the CNT, which (in our simulations) exerted a smaller total force on the lipid–CNT closure. For the same reason, the progression of lipids on the CNT surface also became slower.

The sizes and filling of these kinetically stabilized micelles might be controlled by the CNT diameter and the forces applied on the two sets of molecules. In principle, the micelles should form already at very small velocities of the lipid and hexadecane molecules, which were beyond the reach of our simulations. When we increased the velocity of water (f_1 = 2.78 pN, f_2 = 1.39 pN), we observed the formation of small micelles with only a few hexadecane molecules. When we also increased the velocity of hexadecane, larger micelles with more hexadecane were formed. When the velocities of the hexadecane molecules were too high relative to that of water (f_1 = 1.39 pN, f_2 = 3.77 pN), the slowly moving lipids did not have time to cover the progressing hexadecane molecules, which started to pass through the partially formed micelles at the CNT exit. These results show that kinetics, thermodynamics, and hydrodynamics all play a role in micelle formation, filling, and detachment from the CNT tip.

In order to test the stability of the thus-formed filled molecular assemblies, we separated the three micelles shown in Figure 1d

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and evolved them in an isolated cell (20 nm × 20 nm × 40 nm) as before. As shown in Figure 1e, the micelles were fully stable for 400 ns. In reality, the micelles could be stable for much longer times, during which they could be applied in various ways.

We compared these kinetically stabilized micelles with micelles formed by thermodynamic stabilization from hydrated lipid and hexadecane monomers. To do so, we heated the system in Figure 1e for a short time, until the micelles disintegrated into their hydrated components, i.e., 305 biheaded lipids (concentration of 3.8×10^{-2} M) and 206 hexadecane molecules. Next, we cooled the system back to T = 350 K and simulated it as before. We observed that within 10 ns, the lipids formed many small micelles of different sizes, filled with random numbers of hydrophobic hexadecane molecules. Within 20 ns, all of the hexadecane molecules were located inside the micelles. Later, smaller micelles merged and formed larger micelles, as shown in Figure 1f for 350 ns long simulations. The sizes of the formed micelles and the numbers of hexadecane molecules stabilized inside them fluctuated significantly in these simulations. In larger volumes, the micelle parameters should stabilize at longer times and depend on the used molecules and solution. Experimental preparation of such thermodynamically stabilized filled micelles allows only limited variation of the types and numbers of molecules present inside the micelles. In contrast, the outlined approach allows controlled self-assembly of kinetically stable micelles with variable parameters.

To further extend these ideas, we also tested the self-assembly of hexadecane-filled micelles formed by single-headed lipids. In solutions, similar lipids (alone) at concentrations above the CMC tend to form tubular structures called bicelles,[14] or planar bilayers,[14] as a result of the limited steric strain caused by the absence of the second hydrophilic head.[14] We prepared a system formed by 1000 lipid molecules adsorbed on the (40,0) CG CNT and 400 hexadecane molecules filling the CNT. The simulation conditions were the same as before.

In Figure 2a, we can see that after 20 ns of equilibration, these single-headed lipids cylindrically wrapped around the CNT. Once the self-assembled lipids were dragged by the flowing water, the CNT tip was covered within 5 ns by the first cylindrical hemimicelle. This time, a filled tubular bicelle was formed. About 20 ns later, a bicelle with $d \approx 3$ nm and length $l \approx 20$ nm was filled with ≈200 hexadecane molecules. As a result of initial fluctuations of the lipid density, the bicelle was eventually cut off and detached from the CNT tip, after which the formation of the second bicelle continued.

Although these lipids seemed to self-assemble into bicelles, it was not clear whether the molecular assemblies would retain their elongated shape. To estimate their stability, we separated the bicelle formed Figure 2d and evolved it in an isolated 20 nm × 20 nm × 37 nm cell as before. We observed that the bicelle was stable for 30 ns, at which point it started to gain a spherical shape. Eventually, a large filled micelle was formed, as shown in Figure 2e for 375 ns long simulations. These results indicate that single-headed lipids with the right structure might assemble on CNTs into kinetically stable bicelles. Using the described methodology, we could potentially control the sizes and filling of these kinetically stable molecular assemblies of single- and double-headed lipids.

As in the previous case, we tested the thermodynamic stabilization of the filled micelles from the hydrated lipid and hexadecane monomers. We heated the hydrated micelle in Figure 2e until it disintegrated into 490 single-headed lipids (concentration of 7.2×10^{-2} M) and 184 hexadecane molecules. Next, we cooled the system back to T = 350 K and simulated it. Within 10 ns, the lipids formed small micelles of different sizes, and in the next 20 ns, all of the hexadecane molecules filled them. Eventually, smaller micelles merged and formed bigger micelles, as shown in Figure 2f, which was obtained for 450 ns simulations. Again, the micelle sizes and the numbers of hexadecane molecules inside them fluctuated significantly in these relatively short simulations. The parameters might later fluctuate less, but their absolute values could not easily be controlled in thermodynamic stabilization.

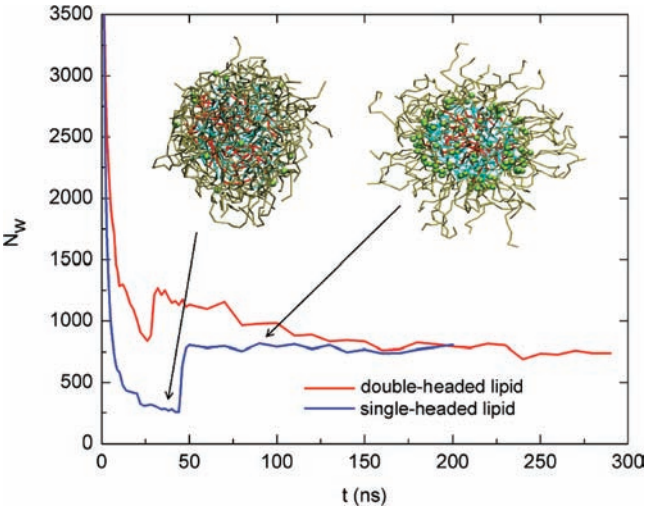

Figure 3. Number of water molecules present within 0.5 nm (first monolayer) of the hydrophobic micelle core during the self-assembly of hydrated double-headed (red) and single-headed (blue) lipid monomers into micelles filled with hexadecane. The inset shows different forms of one micelle selected from the system of self-assembling single-headed lipid monomers.

To test the equilibration of these micelles self-assembled from the two kinds of lipid monomers (Figures 1f and 2f), we calculated the total numbers of water beads (N_w) within a distance of 0.5 nm (first layer) from their hydrophobic cores. Figure 3 shows that with progressing self-assembly, N_w initially largely decreased in both systems from the initial value of $N_w \approx 7000$. In the single-headed (double-headed) lipid system, N_w approached a minimum at 40 ns (25 ns) due to initial hydrophobic collapse[35] of the lipids. Next, the micelles started to swell, and some water beads reached the hydrophobic surface of the core. Eventually, the numbers of water beads (accessible surface) at the micelle cores stabilized, and the systems equilibrated.

In summary, we have shown that kinetically stable micelles filled with molecules that are poorly soluble in water could be prepared on CNT surfaces. The sizes and filling of the formed micelles could be controlled by the preparation conditions, such as the sizes of the nanotubes and the preparation speed. The lifetimes of the formed micelles depend on their size, the monomers used, the molecules carried, and the solution. Other nanostructures might also potentially be used in the controlled self-assembly of filled micelles. The outlined methodology has potential applications in molecular storage, protection, manipulation, and delivery.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Simulation methods and two movies related to micelle formation from double-headed lipids

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and bicelle formation from single-headed lipids (AVI). This material is available free of charge via the Internet at http://pubs.acs.org.

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