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Placing and shaping liposomes with reconfigurable DNA nanocages

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Abstract

The diverse structure and regulated deformation of lipid bilayer membranes are among a cell's most fascinating features. Artificial membrane-bound vesicles, known as liposomes, are versatile tools for modeling biological membranes and delivering foreign objects to cells. To fully mimic the complexity of cell membrane and optimize the efficiency of delivery vesicles, controlling liposome shape (both statically and dynamically) is of utmost importance. Here we report the assembly, arrangement, and remodeling of liposomes with designer geometry: all of which are exquisitely controlled by a set of modular, reconfigurable DNA nanocages. Tubular and toroidal shapes, among others, are transcribed from DNA cages to liposomes with high fidelity, giving rise to membrane curvatures present in cells yet previously difficult to construct *in vitro*. Moreover, the conformational changes of DNA cages drive membrane fusion and bending with predictable outcomes, opening up opportunities for the systematic study of membrane mechanics.

Cells have evolved sophisticated mechanisms to regulate membrane shape and dynamics¹. In the past few decades, scientists and engineers developed methods to generate artificial vesicles, or liposomes, as both model systems to study cell biology and drug carriers to interfere with cell behaviour^{2,3}. The geometry of a liposome defines its physical and chemical properties (fusogenicity, binding affinity to proteins, susceptibility to enzymatic modifications, etc.), and therefore needs to be carefully controlled for each specific study and application. While existing techniques are capable of generating size-defined spherical liposomes and certain aspherical vesicles by means of bottom-up lipid assembly and mechanically distorting membranes^{4–10}, design and experiment restraints (for example, lipid

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Author contributions

Z.Z. initiated the project, designed and carried out most of the experiments, analyzed the data, and prepared most of the manuscript. Y.Y. performed cryo-EM study and prepared the manuscript. F.P. modeled energy input for membrane fusion and prepared the manuscript. M.L. performed tomography study, analyzed the data, and prepared the manuscript. C.L. initiated the project, designed and supervised the study, interpreted the data, and prepared the manuscript. All authors reviewed and approved the manuscript.

Competing financial interests

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composition^{11–15}) often limit a method's adaptability, precision, and programmability. In this work, we take a bioinspired approach (namely DNA nanotemplating) to design, build, and change liposome shapes in a programmable, deterministic manner. Unlike previous methods that rely on trial and error to tune vesicle shape, here we directly programed the geometry of a liposome into its DNA template.

Rapid development of DNA-origami technique has led to the construction of DNA structures with increasing complexity and size^{16–18}. Here we adapted several techniques (e.g., caDNAno¹⁹, curved multi-helix bundle²⁰, shape complementarity²¹, flexible connection between stiff components²²) to design DNA-origami nanocages as templates to guide liposome formation. A monomeric DNA nanocage is typically a cylindrical frame, which consists of two terminal rings separated by four parallel pillars and is made from a long single-stranded DNA (ssDNA) derived from M13 bacteriophage genome (scaffold strand) and many synthetic oligonucleotides (staple strands). Selected staple strands carry ssDNA extensions (handles) pointing to the center of the ring for the hybridization with complementary DNA strands (anti-handles) modified with lipid. Two groups of shape complementary two-helix bundle protrusions (teeth) decorate the front and rear ends of each monomer to facilitate oligomerization in the presence of linker strands. This is a modular design: the ring size, the pillar length and rigidity, as well as the number and position of the handles and teeth are all tunable, which allows for the construction of different frames using the same design principle (Fig. 1a).

Results and discussion

One-dimensional liposome array

We assembled liposomes within DNA nanocages following a previously established protocol with minor modifications^{23, 24}. In brief, lipidated anti-handles (a lipid-DNA conjugate between 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[4-(p-maleimidophenyl)butyramide] or 18:1 MPB-PE and thiol-modified DNA oligonucleotide) were first hybridized to the DNA rings in the presence of detergent; excessive lipids were then added followed by dialysis (see Supplementary Table 1 for lipid compositions used in this study). During detergent removal, the lipid molecules on the DNA rings nucleate the formation of liposomes, while the 3D DNA frame defines the final liposome shape. In contrast, classic “template-free” reconstitution generated only spherical liposomes with considerable heterogeneity (Supplementary Fig. 1). Finally, templated liposomes were enriched using isopycnic centrifugation and characterized by transmission electron microscopy (TEM). Experimental procedures are summarized in Figure 1a and described in Supplementary Materials and Methods.

To test the structural integrity and rigidity of our DNA nanocage and its ability to template liposomes, we first built a DNA structure with two 49 nm (inner diameter) rings and four 120 bp (40 nm) four-helix bundle (4hb) pillars (Fig. 1b and Supplementary Fig. 2). Upon the addition of linker strands, rate-zonal centrifugation purified monomers²⁵ polymerized at elevated Mg^{2+} concentration (Supplementary Fig. 3) to form tubes with average length of $0.9 \pm 0.3 \mu m$ (Fig. 1b and Supplementary Fig. 4). While currently the tube length is not a feature by design, in principle “capping” monomers without either front or rear teeth could

be used to tune polymer length. We built two versions of tube to template liposome assembly — one with lipid seeds on every ring and the other with seeds only on every other ring. TEM images clearly indicated the formation of one-dimensional (1D) liposome arrays in both versions (Fig. 1b, 1c, and Supplementary Fig. 4), with inter-liposome distances consistent with the designed values (Fig. 1d). In both versions of tube, at least 87% of the DNA rings with lipid seeds ended up carrying a liposome containing ~77% 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 11% 18:1 MPB-PE, 9% 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (DOPS), and 3% 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG-2k-PE) in TEM images (Supplementary Table 2). Cryo-electron microscopy (cryo-EM) study further confirmed that the templated liposomes were spherical and unilamellar (membrane thickness = 4.3 ± 0.5 nm).

Interestingly, liposome diameters appeared smaller when measured by cryo-EM (28 ± 4 nm) than by negative-stain TEM (36 ± 4 nm). However, both electron microscopy methods reported essentially the same intervals between adjacent liposomes (Fig. 1d), suggesting that the vesicle dehydration and structural distortion (e.g., flattening) during EM sample preparation affected only the radial and not the axial dimensions of the DNA tube and liposome array (further discussed in Supplementary ‘Technical Notes’).

Width-controlled membrane tubules

After demonstrating the DNA tube’s ability to control inter-liposome distance, we hypothesized that if the lipid modified rings were sufficiently close, instead of well-separated liposomes, a continuous membrane tubule would form as a result of the templated lipid self-assembly. To test this, we folded the 49 nm DNA cage without the staple strands in any pillar, leaving the two rings connected only by four groups of unpaired scaffold strands. Consistent with our hypothesis, this led to DNA tubes with densely packed rings (average length = 1.0 ± 0.6 μ m) and the eventual formation of membrane tubules that were homogenous in width (Fig. 2a, middle row and Supplementary Fig. 5). The average diameter (35 ± 5 nm, measured from cryo-EM images) agreed with the theoretical value calculated from a cartoon model (35 nm, cf. Fig. 2b), where the lipid modified DNA duplexes were considered as rigid rods. It is worth noting that the DNA template is not designed to confine the terminals of the lipid tubule. As a result, although the lipid tubules often ended with hemispherical caps with the same base diameter as the tubule width, some tubules carried much larger vesicles at either end (Supplementary Fig. 5 and 6), appearing like the vesicle budding necks in cells.

To test the versatility of the DNA tubes in templating width-controlled membrane tubules, we built two more DNA nanocages with internal widths of 66 nm and 28 nm. The 66 nm cage has a cylindrical shape with two rings separated by four 42 bp (14 nm) 4hb pillars, and it was able to form long tubes up to 5 μ m or ~150 monomers (average length = 2.1 ± 1.3 μ m) in the presence of linker strands (Fig. 2a, bottom row). The 28 nm cage consists of two squares connected by four groups of unpaired scaffold strands. After polymerization, these narrow cages formed tubes with average length of 1.0 ± 0.5 μ m, albeit with greater flexibility than the 49 nm and 66 nm tubes (Fig. 2a, top row). After DNA-frame guided lipid self-assembly, membrane tubules with homogenous width formed inside both 28 nm and 66 nm

tubes, as shown in Figure 2a, S6 and S7. Cryo-EM studies confirmed that the membrane tubule diameters agreed well with predicted values (Fig. 2b). On average, the fraction of 28, 49, and 66 nm DNA tubes filled with membrane tubules were 69%, 95%, and 99%, respectively (Supplementary Table 2). Among the three types of DNA tube, the 66 nm tube resulted in longest and straightest membrane tubules, consistent with the template's highest stiffness and greatest length. Comparing with the methods that generate membrane tubules by tuning lipid compositions^{12–15} or mechanically pulling on giant vesicles^{26, 27}, an enticing feature of the DNA-templated liposome formation is that the lipid assembly outcomes depend less on the liposomes' chemical makeup or membrane tension²³. Figure 2 shows the membrane tubules made from ~80% DOPC, 11% 18:1 MPB-PE and 9% DOPS. To test the compatibility between lipids' chemical structure and liposome shape, we varied lipid compositions to include phospholipids with different head group (size and charge) and fatty acid chains (length and state of saturation) as well as cholesterol (Supplementary Table 3). The vast majority of the lipid compositions that we tested had negligible effect on the width of lipid tubules templated by 28, 49, and 66 nm DNA tube; the products were indistinguishable under TEM (Supplementary Fig. 5–7). However, PEG-2k-PE disrupted the formation of membrane tubules inside 28 nm DNA tubes and led to 1D arrays of discrete liposomes (diameter = 18.4 ± 2.4 nm), possibly because the steric coating stabilized individual vesicles and prevented their further merger during reconstitution (Supplementary Fig. 7). Together, the formation of lipid tubules with three different predefined widths proved the programmability of the DNA nanocages and the feasibility of generating templated aspherical liposomes.

Liposomes with complex curvatures

In pursuit of more complex membrane curvatures, we took advantage of the design modularity and further reengineered the DNA nanocages to generate three more shapes: torus, helix, and stacked tube. First, because the length of each pillar in a nanocage can be independently tuned, we were able to introduce curvature to the 49 nm and 66 nm DNA cages by making the pillars on one side of the cage shorter than the others (Supplementary Fig. 8). In the case of the 49 nm cage, we designed the length of three pillars to be 64, 32, and 32 bp, while leaving the fourth pillar completely unfolded. This theoretically resulted in a monomeric fan-shaped nanocage with 23° of curvature, which could oligomerize into a donut-like structure as validated by TEM (Fig. 3a, left). Similarly, by leaving two neighboring pillars of the 66 nm cage unfolded, we built a larger donut-like DNA frame (Fig. 3a, right). Moreover, these DNA donuts were capable of guiding the formation of membrane tori inside with a variety of lipid compositions (Supplementary Fig. 9). Liposomes shown in Figure 3a were made from ~77% DOPC, 11% 18:1 MPB-PE, 9% DOPS, and 3% PEG-2k-PE. Cryo-EM images showed the continuous double bilayers of the tori encompassed by their DNA templates (Fig. 3a and Supplementary Fig. 10). We noted in negative-stain TEM images that the measured major radii of both membrane tori (50 ± 6.1 nm, N=72 and 73 ± 7.6 nm, N=31) were significantly smaller than the corresponding theoretical values (67.3 nm and 125 nm) while their measured minor radii (22 ± 1.8 nm, N=72 and 33 ± 4.2 nm, N=31) matched fairly well with the designs (17.5 nm and 26 nm) considering the slight TEM sample deformation (Supplementary Fig. 11). Cryo-EM study gave similar measurements. We therefore speculated that most toroidal liposomes formed in

these experiments had a modest global twist (Supplementary Fig. 11), although they were topologically equivalent to tori and homogenous in size. Cryo-EM tomography on a membrane torus confirmed the topology of the liposome and revealed its uneven top and bottom surfaces, supporting this hypothesis (Supplementary Fig. 12). The toroidal liposomes formed with lower yield (9% in 49 nm cage and 23% in 66 nm cage) than their cognate tubular liposomes, likely due to the defects in DNA template. For example, improperly closed DNA donuts can lead to curved but open-ended membrane tubules, which were in fact a major byproduct (Supplementary Fig. 9). Importantly, these nanoscale lipid tori have ever-changing membrane curvature along the poloidal direction and resemble the membrane topology surrounding a vesicle fusion pore or nuclear pore complex.

Next, we shifted the teeth on the front ring of a fan-shaped 66 nm DNA cage by 11° clockwise to force a left-handed twist during polymerization. TEM images confirmed the assembly of helical DNA cages and the templated formation of membranous springs with expected helical pitches and diameters at a yield of ~36% (Fig. 3b and Supplementary Fig. 13). Finally, in addition to the teeth located at the front and rear faces, we built teeth on two opposite sides of a cuboidal DNA cage, which enabled linker-strand mediated stacking in both lateral and axial directions. The resultant DNA-templated lipid tubule arrays (Fig. 3c and Supplementary Fig. 14) remotely resembled the shape and topology of Golgi cisternae.

DNA-cage controlled membrane dynamics

Now that we have established that static DNA cages can template various biomimetic membrane structures, it is tempting to use dynamic DNA templates to change liposome shapes. Although liposome fusion has been mediated by ssDNA tethers^{28, 29}, a more versatile and rigorously controlled system is desirable for the *in vitro* study of membrane mechanics (see Technical Note in SI for more discussions). To prove this concept, we performed two types of structural reconfiguration on our DNA nanocages to drive membrane fusion and bending. For membrane fusion, we intended to bring the parallel DNA rings in a 1D liposome array into close proximity. To implement this idea, we folded the pillars of 49 nm DNA tubes with staple strands displaying 6 nt overhangs (toeholds); the complete removal of these strands by toehold-mediated strand displacement³⁰ transformed the 40 nm 4hb pillars to ssDNA loops, which acted as entropic springs to bring close the neighboring DNA rings (Fig. 4a and Supplementary Fig. 15). As we had expected, disassembling the DNA pillars in a templated 1D liposome array pulled the spherical liposomes (~77% DOPC, 11% 18:1 MPB-PE, 9% DOPS, and 3% PEG-2k-PE) into contact and fused many of them into lipid tubules (Fig. 4b and Supplementary Fig. 15). We carried out three control experiments to ensure that the membrane fusion was mediated by the DNA cage reconfiguration. First, adding water or non-relevant DNA strands to the liposome arrays did not make any apparent change in liposome shape or inter-liposome distance. Second, dismantling an increasingly longer section (e.g., 1/4, 1/2 or 3/4) of all DNA pillars led to increase of fusion probability (Fig. 4b, Supplementary Fig. 16 and 17). Third, disassembling all pillars of a DNA tube where liposomes were placed on alternate rings resulted in closely packed liposomes without fusion due to the “cushion space” provided by an empty ring between adjacent liposomes (Supplementary Fig. 18). Further, the well-controlled liposome size and DNA pillar configurations allowed for quantitative modeling of the force exerted by

the ssDNA³¹ (Supplementary Methods). Using this model, we derived that up to $\sim 70 k_B T$ was given to each pair of liposomes, on par with the energy requirement for a typical fusion event^{32, 33}.

Similarly, we disassembled two neighboring pillars of the 66 nm DNA tube by toehold-mediated strand displacement to bend templated membrane tubules containing $\sim 80\%$ DOPC, 11% 18:1 MPB-PE, and 9% DOPS. Nearly all lipid-free DNA templates transformed from straight to curved tubes (Fig. 4c and Supplementary Fig. 19) upon the addition of displacing strands. Such structural reconfiguration worked effectively on the templated liposomes, bending 74% of them to a mean inner radius of curvature of 60 nm (Fig. 4d and Supplementary Fig. 19). Interestingly, the other 26% of lipid tubules remained straight (defined as radius of curvature above 1 μm at all places), which may be attributed to defective DNA pillars (Supplementary Fig. 20). In other words, a portion of DNA tubes may have suffered pillar deformation during liposome formation, which did not affect the width of lipid tubules but prohibited their programmable bending.

Discussion and outlook

The power of DNA nanotechnology in creating molecular geometry offers striking opportunities for the fabrication of shape-controlled materials at nanometer and micrometer scales^{34–38}. Harnessing such engineering power to manipulate membrane provides a solution to the long-standing challenge of controlling liposome shape, arrangement, and dynamics. In this work we showed that programmable DNA templates can mold membranes with different lipid compositions into biologically relevant shapes and stabilize them (Supplementary Fig. 21). Although here all liposomes formed at room temperature, lipids with higher transition temperature (e.g., 100% 1,2-dipalmitoyl-sn-glycero-3-phosphocholine) are in theory amenable to our method thanks to the thermal stability of the DNA-origami tubes (stable at 44 °C and could be improved by chemical crosslinking³⁹). In addition, the possibility of phase separation⁴⁰, a biologically important phenomenon where lipid composition and arrangement change locally in bilayers, remains to be explored on the DNA-templated liposomes. While further development awaits (for example, scale up the production for bulk applications), we expect this DNA-nanostructure controlled membrane engineering technique to constitute an enabling platform for the future quantitative study of membrane biophysics and membrane protein structure and function⁴¹ (see Supplementary ‘Technical Notes’ for more discussion). For example, the DNA-cage controlled liposome fusion device could be adapted to study the energy landscape and identify intermediates of spontaneous and protein-mediated vesicle fusion^{42, 43}. Many membrane-associating proteins, such as dynamin^{44, 45}, endosomal sorting complexes required for transport^{46, 47}, and autophagy-related proteins^{48, 49}, can be deployed to the biologically relevant membrane structures generated here to study the curvature-dependent protein-membrane interactions. Further, the membrane integrity of shape-controlled liposomes is evidenced by their impermeability to iodixanol (Supplementary Materials and Methods), calcium, and Fluo-4 (Supplementary Fig. 22), suggesting the liposomes’ potential utility as containers for chemical reactants. With functional protein complexes incorporated, it is possible to engineer vesicles that readily cross cell barriers (e.g., plasma membrane, endosome, and nuclear envelope) and even make artificial organelles.

Methods

Preparation of lipidated DNA anti-handles

Thiol-modified DNA oligonucleotides (Integrated DNA Technologies) were treated with tris(2-carboxyethyl)phosphine (Sigma-Aldrich) for 30 minutes (50 nmole DNA per reaction) and immediately reacted with 1 μ mole of 18:1 MPB PE (Avanti Polar Lipids) in aqueous solutions containing ~2% Octyl β -D-glucopyranoside (OG, purchased from EMD Millipore) at room temperature for 30 minutes. Such a reaction mixture was then diluted ~3-fold with OG concentration brought up to 4%, added to 2.5 μ mole of pre-dried DOPC, agitated, and dialyzed overnight. The conjugation products were separated from unconjugated DNA via isopycnic centrifugation in iodixanol gradients (Cosmo Bio USA) and later analyzed by SDS-polyacrylamide gel electrophoresis.

DNA origami design and preparation

Monomeric DNA-origami cages were designed using caDNAno (cadnano.org). Inner and outer-handle sequences (21-nt) were generated by NUPACK (nupack.org) and manually added to the 3'-ends of the appropriate staple strands. Linker strands were designed such that each 16-nt (or 18-nt) linker uses 12 bases for hybridization with a DNA-origami monomer and the remaining 4 (or 6) bases for binding with another monomer to facilitate front-to-rear oligomerization. DNA scaffold strands (8064-nt) were produced using *E.coli* and M13-derived bacteriophages⁵⁰. Staple strands were synthesized by Integrated DNA Technologies. The DNA cage monomers were assembled from a scaffold strand (50 nM) and a pool of staple strands (300 nM each) in 5mM Tris HCl, 1 mM EDTA, pH 8.0 with 12.5 mM of MgCl₂ using a 36-hour thermal annealing program. Correctly assembled DNA cages were purified *via* rate-zonal centrifugation in glycerol gradients as described previously²⁵. To make DNA-origami oligomers, linker strands were added to purified monomers at a 20:1 molar ratio. The mixture was then annealed from 40°C to 20°C overnight in a solution containing 30 mM MgCl₂ and used without further purification.

Preparation of DNA origami templated liposomes

DNA cages were first labeled with lipidated anti-inner-handles and Cy5-modified anti-outer-handles in one-pot hybridization mixtures containing 1% OG. To form DNA-cage templated liposomes, rehydrated lipid mixtures (compositions listed in Supplementary Table 1) were added to lipid- & Cy5- labeled DNA cage at 100,000:1 lipid-to-cage ratio. The solution was diluted to 100 μ L in 1 $\times$ hydration buffer (25 mM HEPES, 400 mM KCl, 30 mM MgCl₂, pH 7.4) with 1% OG, gently shaken for 30 minutes at room temperature, put into a 7kD molecular weight cut-off dialysis cassette (Thermo Scientific), and dialyzed against ~2 L of 1 $\times$ hydration buffer overnight. The dialyzed solutions were subjected to isopycnic centrifugation in iodixanol gradients for liposome purification.

Structural reconfiguration

Structural reconfiguration was initiated by adding appropriate displacing oligonucleotides in 30–60 $\times$ molar excess. The mixture was incubated at 44°C overnight and characterized by

negative-stain TEM without further purification. TEM images were analyzed manually in ImageJ (National Institutes of Health).

Electron microscopy

Negative staining was achieved by adsorbing ~5 μ l sample on a glow-discharged formvar/carbon-coated copper grid (Electron Microscopy Sciences) and staining with 2% uranyl formate. Imaging was performed on a JEOL JEM-1400 Plus microscope operated at 80 kV. For cryo-EM imaging, iodixanol gradient fractions loaded onto a glow-discharged holey carbon grid (Quantifoil Micro Tools) immediately after hyperhydration treatment. The grid was then blotted, flash frozen in liquid ethane, and examined using an FEI Tecnai-F20 electron microscope operated at 200 kV. For tomography study, 5 nm Au nanoparticles (fiducial markers) were pre-mixed with the sample suspension prior to deposition on the grid. A tilt series was recorded for each structure at tilt angles from -60 to 60 degrees with a 2 -degree increment. At each tilt angle, a movie was recorded with a Gatan K2 camera. The drift-corrected tilt series were processed to generate tomograms using IMOD (University of Colorado).

Liposome-leakage assay

DNA-cage templated liposomes were prepared with 50 mM CaCl_2 in the hydration buffer. Free Ca^{2+} were removed from purified liposomes by dialysis. Fluo-4 (Thermo Fisher Scientific) were added to the dialyzed liposomes to achieve a final concentration of $2 \mu\text{M}$; background fluorescence was suppressed by adding EDTA (final concentration: $30\text{--}210 \mu\text{M}$). Fluorescence (excitation/emission: $480/520 \text{ nm}$) of this solution was then recorded for 30 minutes before and after the addition of 1% OG.

Data availability

DNA-origami designs and electron micrographs are available within the Article and its Supplementary Information, or from the corresponding author upon reasonable request.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Organizing liposomes with DNA nanocages. (a) Main panel: general concept of DNA-cage controlled formation and remodeling of liposomes. The geometry, chemical modification, and structural reconfiguration of DNA cages dictate the shape and dynamics of templated liposomes. Inset box: reagents and procedures used in each step of liposome preparation. Cartoon models in step 1 show the polymerizing interface where teeth (red box) of two monomers were brought together by DNA linkers (red arrows). Note that without the linker strands, the teeth are incomplete (with unpaired ssDNA loops) and thus incapable of

stacking. Cartoon models in step 2 show a top view of a lipid-seed decorated DNA tube (bottom). (b) Assembly of one-dimensional liposome array. From left to right: cartoon models and electron micrographs of a monomeric DNA cage, polymerized DNA cages, and liposome arrays as a result of templated liposome formation. Insets are zoomed-in cryo-EM images. (c) Placing lipid seeds on every other ring (dark blue) of the DNA tube resulted in liposome arrays with doubled inter-liposome distances. (d) Inter-liposome distances in liposome arrays measured from cryo-EM and negative-stain (n.s.) TEM images compared with theoretical values. Liposomes contain ~77% DOPC, 11% 18:1 MPB-PE, 9% DOPS, and 3% PEG-2k-PE. Error bars represent standard deviations (N=78–237). Scale bars: 100 nm.

Figure 2.

Templated assembly of width-defined membrane tubules. (a) Membrane tubule formation inside 28 nm (top), 49 nm (middle), and 66 nm (bottom) DNA tubes. Each row shows cartoon models and electron micrographs of DNA cage monomers (left), polymers (middle), and DNA-tube templated membrane tubules (right). Insets show close-up views of DNA templates under negative-stain TEM and DNA-templated liposomes under cryo-EM. Scale bars: 100 nm. (b) The diameters of membrane tubules shown in (a) compared with theoretical values. Liposomes contain ~80% DOPC, 11% 18:1 MPB-PE, and 9% DOPS.

Cartoon models are shown in top view to illustrate the predicted membrane tubule width. Error bars represent standard deviations (N=15–22).

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Figure 3.

Generating membrane structures with complex curvature. (a) Two types of toroidal liposomes formed inside donut-shape DNA templates. First row from the top: cartoon models of DNA donuts (R : major radius; r : minor radius). Second row: negative-stain TEM images of DNA templates. Third and fourth rows: negative-stain TEM and cryo-EM images of membrane tori. (b) Helical liposomes formed inside spring-like DNA templates. Cartoon models are shown on the top; negative-stain TEM images of DNA templates and liposomes are shown on the bottom left and right, respectively. (c) Lipid tubule arrays templated by DNA tube stacks. Top row: Cuboidal DNA cages with additional sets of teeth (yellow) can polymerize in both y - and z -directions. Bottom row: negative-stain TEM images of DNA

tube stacks (left) and lipid tubule arrays (right). Liposomes in (a) and (b) contain ~77% DOPC, 11% 18:1 MPB-PE, 9% DOPS, and 3% PEG-2k-PE; stacked lipid tubules in (c) contain ~80% DOPC, 11% 18:1 MPB-PE, and 9% DOPS. Scale bars: 100 nm.

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Figure 4.

Dynamic control of liposome shape by DNA template reconfiguration. (a) Reducing the inter-ring distance in a DNA tube by disassembling all its pillars. Insets show the strand diagrams of a short stretch of pillar before and after toehold-mediated strand displacement. (b) Fusing liposomes in a 1D array (~77% DOPC, 11% 18:1 MPB-PE, 9% DOPS, and 3% PEG-2k-PE) through the structural reconfiguration shown in (a). Liposome fusion probability (defined as fusion events divided by the sum of fusion and non-fusion events) in different experiment setups are shown as bar graphs. Displacement strand set (disp. set) n disassembles $n/4$ of each pillar. In every set-up, there were 135–301 total events observed. (c) Bending a DNA tube by disassembling all its pillars on one side. (d) Bending lipid tubules (~80% DOPC, 11% 18:1 MPB-PE and 9% DOPS) through the structural reconfiguration shown in (c). All electron micrographs are captured by negative-stain TEM. Scale bars: 400 nm.