Membrane sculpting by curved DNA origami scaffolds

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Membrane sculpting and transformation is essential for many cellular functions, thus being largely regulated by self-assembling and self-organizing protein coats. Their functionality is often encoded by particular spatial structures. Prominent examples are BAR domain proteins, the ‘banana-like’ shapes of which are thought to aid scaffolding and membrane tubulation. To elucidate whether 3D structure can be uncoupled from other functional features of complex scaffolding proteins, we hereby develop curved DNA origami in various shapes and stacking features, following the presumable design features of BAR proteins, and characterize their ability for membrane binding and transformation. We show that dependent on curvature, membrane affinity and surface density, DNA origami coats can indeed reproduce the activity of membrane-sculpting proteins such as BAR, suggesting exciting perspectives for using them in bottom-up approaches towards minimal biomimetic cellular machineries.
The curvatures of biological membranes vary strongly, from predominantly flat in the plasma membrane to highly curved in the endoplasmatic reticulum or in the Golgi apparatus. The transformation of membranes from one shape to another, for example during cell division, belongs to the most fundamental processes in living cells. Numerous factors that regulate membrane curvature have been identified, with scaffolding proteins being the most obvious ones. An important class of scaffolding proteins which presumably imprint their shape on lipid membranes is the BAR (Bin/Amphiphysin/Rvs) domain superfamily. When dimerized, BAR proteins form characteristic banana-shaped scaffolds that induce and stabilize membrane curvature through electrostatic and hydrophobic interactions. Several BAR proteins were shown to tubulate membranes in vitro. BAR proteins presumably rely on their curved shape for their activity: different types of BAR modules adopt folds with different degrees of curvature. Taking inspiration from the different degrees of curvature covered by BAR domain proteins, three DNA origami designs (20-helix bundles with hexagonal lattice; Supplementary Figs. 2–4 and Supplementary Tables 1–3) were here developed (Fig. 1): (i) a ‘semi-circle’ named HALF (origami H) with curvature \( C \approx 21.7 \mu m^{-1} \); (ii) a ‘quarter-circle’ named QUARTER (origami Q) with \( C \approx 11.6 \mu m^{-1} \) and (iii) a ‘stick’ named LINEAR (origami L) with \( C = 0 \) (Fig. 1a–c and Supplementary Fig. 5). Despite their fivefold increased length when compared to BAR proteins (~110 nm vs. ~20 nm, respectively), these origami structures (H, Q and L) mimic the typical shapes of highly-curved BAR/N-BAR dimers, moderately curved F-BAR dimers and flat PinkBAR/I-BAR dimers.

Programmable self-assembly with DNA origami may be employed to produce a variety of two-dimensional and three-dimensional structures on the nanometer-scale, including objects with custom curvature. This molecular toolkit now serves as the starting point for our goal of constructing membrane-sculpting machinery from the bottom-up. DNA origami has been previously employed to create nanoscale channels in lipid membranes and to guide the assembly of nanoscale lipid compartments. In contrast to DNA origami nanocages that template small liposomes via detergent removal, our designed origami structures act on preexisting cell-sized vesicles, imitating the mechanism of action of protein coats. Subsequently, in this work, we achieve the transformation of membrane shape on much larger scales, reminiscent of deformations observed in cells.

**Fig. 1** BAR-mimicking DNA origami nanoscaffolds. **a** Structures of origami L (linear), Q (quarter) and H (half), which mimic the shape of I-BAR, F-BAR and BAR/N-BAR domains, respectively. **b** Corresponding negative-stain TEM images of the folded curved nanostructures. **c** The angle of curvature and respective radius of origami structures Q and H (84 and 46 nm, respectively) were experimentally determined from TEM images \( n = 110-130 \). **d** Schematic representation of marked positions on the top convex (T0–T7), bottom concave (B0–B7), lateral sides (L0–L13, R0–R13) and tips used on the nanoscaffolds (here origami Q) for attaching fluorophores, membrane-anchoring moieties or oligomerizing staples. Scale bars: 100 nm.
Results

Efficient binding of curved DNA origami to membranes. We assessed the interaction of curved DNA nanostructures with lipid membranes, mainly giant unilamellar vesicles (GUVs) composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), via fluorescence confocal microscopy (Fig. 2). Incorporation of 7 × Atto488-modified staples on positions T0-6 enabled fluorescence detection of the origami structures (Fig. 1d). Similarly to what was described elsewhere, bare DNA origami structures lacking membrane anchors were adsorbing to lipid bilayers in the presence of 20 mM MgCl2 (Supplementary Fig. 6). We avoided such unspecific membrane attachment (Supplementary Fig. 7) and ensured long-term stability of the nanostructures with an imaging buffer containing 5 mM MgCl2 and 300 mM NaCl in which the Na+ outcompetes membrane-adsorbed divalent cations via a counterion release mechanism to break up Mg2+ promoted interactions between DNA and the phospholipids.

To achieve side-specific binding of the curved DNA origami structures to lipid membranes, we tested various methods including neutravidin-mediated attachment of biotinylated origami H to tetrathiolated lipids (Supplementary Fig. 8a) and covalent attachment of thiolated origami H to maleimide-modified lipids (Supplementary Fig. 8b). However, preferred membrane anchors were oligonucleotides linked to a cholesteryl moiety via a tetraethylene glycol spacer (TEG-chol), as they have already been extensively characterized and allowed for a steady binding of nanostructure H to lipid bilayers (Supplementary Fig. 9) in comparison to the other approaches.

In order to enhance attachment of curved DNA origami scaffolds to membrane models and avoid steric hindrance (Supplementary Figs. 10–12), we placed TEG-chol moieties at the distal 5′-end of 18 bps-long linker sequences extending from the origami backbone (anchor orientation called TC5). Placing the anchors closer to the origami backbone, i.e., at the proximal 3′-end of the linker sequences (Supplementary Fig. 10a and Supplementary Fig. 11d–f), or shortening the linker length from 18 to 9 bps (Supplementary Fig. 12b, d), severely reduced binding of nanostructures H and Q to membranes. This effect was particularly prevalent for membrane binding through the concave origami surface.

Since anchor accessibility plays a decisive role for attaching DNA origami structures to lipid membranes, we further evaluated how number and positioning of TC5 anchors along the concave origami facet may influence binding of nanostructures H and Q to GUVs. When single TC5 anchors were introduced (Supplementary Fig. 13b–d and h–j), no significant attachment of our curved nanostructures to membranes was observed (Supplementary Fig. 14). In contrast, when three TC5 anchors were incorporated (Supplementary Fig. 13e–f and k–l), membrane affinity was significantly increased, especially if the anchors were placed at positions B0, B3 and B6 (combination from here on called X3) (Fig. 2c and Supplementary Fig. 13f, l). Using negative-stain TEM imaging, we further corroborated the attachment of construct Q3 to lipid vesicles (Fig. 2e).
Taken together, we identified some of the major requirements for efficiently attaching curved origami structures to lipid bilayers and we observed indications of curvature-mediated deformations of membranes induced by the F-BAR mimicking structure Q3 (Fig. 2d and Supplementary Fig. 13m). Notably, incubation of GUVs with Q3 for at least 1 h led to the appearance of outwards tubular deformations upon hyperosmotic stress (b, marked by arrows). Similarly, membrane interaction of origami Q variants displaying three cholesteryl anchors on different curved facets was further investigated. Strong binding to GUVs was achieved for all nanostructures, independently of the facet where anchors are localized (d-f). Upon vesicle deflation, the concave structure (Q3) triggered outwards membrane tubules (d, marked by arrows); the convex structure (QI3) triggered evagination/invagination-type of deformation (e, marked by arrows); and the structure with null curvature (QR3) led to no significant changes in vesicle shape (f). Scale bars: 5 µm

Membrane deformations as a function of DNA origami curvature. As different classes of BAR domains curve membranes in distinct manners, we further investigated whether the appearance of membrane deformations, as reported in Fig. 2d, can be correlated with the direction and degree of curvature of our BAR-mimicking DNA origami structures. Membrane tension has been previously implicated in influencing the assembly of BAR domain proteins. To provide a controllable trigger for assessing vesicle deformations, we lowered the membrane tension by increasing osmolarity of the outer buffer in 10%. Subsequently, shape variations of the deflated GUVs were monitored.

After the hyperosmotic stress, lipid vesicles without membrane-bound DNA origami (no origami in solution or incubated with nanostructures lacking cholesteryl anchors) rapidly regained their spherical shape, suffering only minor shrinkage or blebbing (Supplementary Fig. 16h–j and Supplementary Movie 1). Bursting events were seldom (~13%; 5 out of 40 GUVs). For vesicles incubated with a structure lacking curvature (L3), a comparable effect was observed (Fig. 3a and Supplementary Fig. 15i, j), independently of the total DNA origami concentration. Remarkably, moderately curved origami quarter-circles (Q) displaying a concave membrane-binding surface were able to trigger tubulation of GUVs upon hyperosmotic shock (Fig. 3b and Supplementary Movie 2). As seen for structure Q3, this process depended on the total origami concentration. At Q3 concentrations ≤3 nM, most vesicles presented no significant deformations, with only a minor fraction (~18%) displaying outwards tubules (43 out of 244 GUVs). By contrast, at Q3 concentrations ≥5 nM, a significant fraction of vesicles (~18%; 22 out of 121 GUVs) displayed outwards tubular deformations within structures upon hyperosmotic stress (Fig. 2d and Supplementary Movie 1).

![Fig. 3](image-url)
Hierarchical oligomerization of curved DNA origami scaffolds. Self-assembly of membrane scaffolding proteins into higher-order structures was suggested to play an important role in the mechanism of action of BAR domains. Both lateral and tip-to-tip linear intermolecular interactions were described to stabilize their assembly into protein lattices. To test the influence of such higher-order linkages, we designed variants of curved DNA origami Q that could oligomerize, similar to BAR proteins, tip-to-tip (Supplementary Fig. 18b–d) and laterally (Supplementary Fig. 18e). Overall, four constructs capable of multimerizing in solution were created: origami Q-E5 (Supplementary Fig. 18b), Q-E7 (Supplementary Fig. 18c) and Q-E13 (Supplementary Fig. 18d) able to linearly multimerize from the tips forming arc-like oligomers of tunable size; plus origami Q-S14 (Supplementary Fig. 18e) able to multimerize laterally forming sheet-like oligomers. Constructs Q-E5/7/13 possess 2 × 5, 7 and 13 blunt ends at defined helices, enabling intermolecular stacking at the origami tips. Construct Q-S14, on the other hand, displays 2 × 14 TATAA overhangs, enabling complementary lateral interactions along the origami sides.

Subsequently, we tested whether the inclusion of those polymerizing staples would enhance the ability of origami Q3 with concave membrane-binding interface to produce tubular membrane deformations on GUVs upon deflation. Altogether, no significant differences in terms of total bulk concentration required to induce tubulation of vesicles were observed for constructs with or without tip-to-tip oligomerizing staples (i.e., structures Q3-E5/7/13 vs. Q3, respectively; Supplementary Fig. 18g–i and Supplementary Movie 20). In contrast, for the construct with lateral oligomerizing staples (Q3-S14), lower bulk concentrations were required for inducing membrane tubulation upon osmotic stress (Supplementary Fig. 19a–b and Supplementary Fig. 18j). Indeed, ~70% of the vesicles incubated with Q3-S14 presented tubular deformations at concentrations ≥3 nM (135 out of 193 GUVs). Likewise, inclusion of lateral polymerizing overhangs on origami Q3 with convex membrane-binding interface (i.e., Q3-S14) also affected the generation of membrane deformations (Supplementary Fig. 21). Here while most vesicles displayed evagination-type membrane deformations upon hyper-osmotic stress (Supplementary Fig. 21e, f), ~15% of vesicles (36 out of 244 GUVs; at 5 nM Q3-S14) additionally presented inward tubules (Supplementary Fig. 21d, g) resembling protruding nanotubes described for convex I-BAR proteins; which could not be observed for the structure Q3 lacking lateral overhangs. Incubation with lower bulk concentrations of Q3-S14 (i.e., 2 nM), on the other hand, did not promote significant membrane deformations, similar to what was observed for convex structure Q-I3 lacking polymerizing overhangs (Supplementary Fig. 16k).

In summary, our data indicate that in particular the presence of lateral interactions influences the ability of curved membrane-bound DNA origami to deform membranes. However, this effect seems to be of minor significance, as structures having additional membrane anchors but lacking polymerization strands (i.e., Q7; Supplementary Fig. 19i–l), were able to deform lipid vesicles as efficiently (in terms of total origami concentrations required) as the structures with polymerization strands (i.e., Q3-S14).

Membrane density and binding affinity of curved DNA origami. Our results so far strongly suggest that a critical membrane density of curved nanostructures is required for triggering membrane bending. To test this hypothesis, variable surface densities of our BAR-mimicking DNA-based scaffolds to DOPC GUVs were quantitatively investigated at equilibrium (after overnight incubation), by fluorescence imaging and single molecule detection.

Apparent membrane dissociation constants at equilibrium (Kd ± s.d.) were obtained for L3, Q3, and H3 structures by fitting the fluorescence intensity values on the surface of GUVs as a function of bulk concentration to a Langmuir isotherm (equation 1): Kd (L3) = 0.39 ± 0.07 nM (n = 131–157 GUVs per fit, 2 repeats), Kd (Q3) = 0.68 ± 0.18 nM (n = 277 GUVs; n = 83–100 GUVs per fit, 3 repeats) and Kd (H3) = 0.39 ± 0.07 nM (n = 131–157 GUVs per fit, 2 repeats), Kd (Q3) = 0.68 ± 0.18 nM (n = 277 GUVs; n = 83–100 GUVs per fit, 3 repeats) and Kd (H3) = 0.39 ± 0.07 nM (n = 131–157 GUVs per fit, 2 repeats).


**Fig. 4** Tubulation of flat membranes depends on surface density of membrane-bound curved origami Q. Fluorescence intensities of membrane-bound DNA origami (labeled with 3 × Atto-488 dyes) at equilibrium (incubated overnight) were extracted using image analysis and represented as a function of total bulk concentration (a, here depicted for one independent set of measurements with Q3). Representative confocal images at the equatorial plane for membrane-bound Q3 nanostructures are depicted in c. Membrane binding of the DNA nanostructures was quantitatively investigated by fitting the data to a Langmuir isotherm (equation 1), enabling the determination of apparent membrane dissociation constants \( K_d \) (± s.d.); L3 (\( n_{total} = 288 \) GUVs; \( n = 131–157 \) GUVs per fit, 2 repeats), Q3 (\( n_{total} = 277 \) GUVs; \( n = 83–100 \) GUVs per fit, 3 repeats) and H3 (\( n_{total} = 106 \) GUVs; \( n = 48–58 \) GUVs per fit, 2 repeats) (b). \( \Delta G_{bending} \) was calculated via \( \Delta G = RT \ln K_d \). Regarding efficiencies of membrane tubulation (d), high yields (> 80%) were retrieved for Q3 bulk concentrations ≥ 0.5 nM, or, upon conversion to surface densities, for ≥90 membrane-bound DNA origami particles per µm² (as illustrated in c). Scale bars: 10 µm. Error bars in b correspond to s.d.

(H3) = 2.0 ± 0.6 nM (\( n_{total} = 106 \) GUVs; \( n = 48–58 \) GUVs per fit, 2 repeats). Thus, for the same combination of cholesteryl anchors, increasing curvature of the DNA nanoscaffolds from flat (\( C = 0 \)) to highly curved (\( C = 21.7 \mu m^{-1} \)) prompted a fivefold weaker binding to flat freestanding membranes (Fig. 4b and Supplementary Fig. 24).

By image analysis, we further quantified the efficiencies of vesicle tubulation by the curved DNA origami Q3 nanostructures (Fig. 4d; \( n_{total} = 445 \) GUVs, \( n = 78–108 \) GUVs per origami concentration). When compared to the results obtained at a shorter incubation period (Fig. 2c, d and Fig. 3), after overnight incubation lower origami bulk concentrations and no additional osmotic perturbation were required to achieve high yields of membrane tubulation. At equilibrium, >80% of vesicles (154 out of 185 GUVs) presented tubular deformation for Q3 bulk concentrations ≥ 0.5 nM (value close to \( K_d \)). Despite slightly increased membrane affinities (lower \( K_d \) values; Supplementary Fig. 24) when compared to structure Q3, structures with increased numbers of anchors (Q7) or with polymerizing overhangs (Q3–S14) yielded similar membrane tubulation efficiencies (Supplementary Fig. 25).

As the number of fluorescent particles is proportional to the fluorescence intensity, we performed additional FCS measurements in order to calibrate the measured fluorescence values and recover the corresponding densities of membrane-bound DNA origami at the surface of GUVs (see calibration curve in Supplementary Fig. 22). Considering the average fluorescence intensities of single DNA origami structures, for Q3 with moderate curvature, we estimated 50 ± 20 particles per µm² bound to GUVs (\( n = 51 \)) to be sufficient for initiating tubulation, and 90 ± 20 particles per µm² (\( n = 50 \) GUVs) for almost all vesicles (>80%) to present tubules (representative curve depicted in Fig. 4a and confocal images in Fig. 4c). At these surface densities, our curved nanoscaffolds cover 9–16 % of the total membrane surface area. Interestingly, this surface fraction matches the previously reported coverage required for BAR domains to induce membrane deformations on model membranes (2–4× higher than for amphiphysin). Flat structure L and highly curved structure H, on the contrary, were not capable of inducing membrane tubulation on GUVs even at surface coverages ≥100 particles per µm² (Supplementary Fig. 23), promoting at best flaccid membrane deformations analogous to the non-spherical shapes previously reported for flat PinkBAR domains. For structure L3, due to its ‘zero’ curvature, no tubulation was to be expected. For highly curved structures H3 and H7, a simple energetic cost-benefit analysis estimates the apparent free energies of membrane adhesion (\( \Delta G = RT \ln K_d \), –20 \( k_BT \) and –21.5 \( k_BT \), respectively) to be clearly insufficient to allow for membrane bending (38 \( k_BT \)). For the moderately curved origami structure Q3, to the contrary, membrane adhesion (–21.1 \( k_BT \)) is strong enough to compensate for the energetic cost of membrane bending (11 \( k_BT \)), hence enabling tubular deformations to be generated.

Finally, we investigated the ultrastructure of membrane tubules decorated with origami Q3 at high surface densities (i.e., after overnight incubation of GUVs with 5 nM Q3), using cryo-
electron microscopy (cryo-EM). From the confocal images (Fig. 5a), the grown tubules appeared homogenously covered with membrane-bound fluorescently labeled Q3. Further cryo-EM imaging (Fig. 5b) confirmed that the surface of the membrane tubules was densely covered with DNA nanostructures, preferentially aligning perpendicularly to the long axis of the tubular structures. Additionally, the recovered tubular diameter (220 ± 70 nm; ntotal = 35 parallel cross-sections, 4 membrane tubules) was in good agreement with the predictions based on the objects curvature (~170 nm; Fig. 1a–c).

Discussion

This work demonstrates that curvature generation and topological transformation of biological membranes, as required for many cellular functions, can be achieved in a well-controlled fashion by curved synthetic scaffolds made of DNA. The action of these scaffolds may be tuned by varying shape, density, membrane affinity, and the propensity for self-assembly of the scaffolds on membrane surfaces. In contrast to earlier work exploring the deformation of membranes by flat nanostructures46, 47, concerted lateral oligomerization by self-assembly plays only a minor role for the specific membrane transforming activity by curved DNA-based scaffolds. Moreover, in spite of producing larger tubular deformations than BAR domains, our curved structures operate at similar membrane bending energy levels. We have established three main requirements for the induction of tubular membrane deformations (Fig. 5c) by scaffolding elements: curvature, membrane affinity and surface density. Remarkably, we provide direct proof that the curvature of membrane associating macromolecular objects plays a decisive role, helping us understand the minimal physical–chemical laws underlying membrane deformations.

In this manuscript, we validate the usage of custom-designed DNA origami as a tool to overcome the limited predictability of engineered proteins. The ability of our developed structures to precisely control local membrane curvature will have great impact in the investigation of all kinds of biological membrane shaping phenomena. For example, sequential binding of proteins involved in deformation cascades (e.g., clathrin-mediated endocytosis48, FtsZ-mediated bacterial division49) depends on the degree of curvature locally displayed by membranes. In this regard, BAR-mimicking DNA origami scaffolds could allow detailed investigation of such proteins on model membranes or even cells50 as a function of local curvature.

Altogether, our work has great significance for the growing field of bionanoeengineering, opening up an avenue of research in synthetic biology. Our present achievements add exciting perspectives towards minimal biomimetic cellular machineries, involved in membrane shaping and beyond; pushing the limits of nanotechnology into cellular biology. As we laid down new foundations on manipulating DNA origami in lipid environments, design of even more elaborate DNA origami supramolecular assemblies targeting lipid membranes (e.g., artificial clathrin coated pits, enzymatic membrane complexes), and novel approaches for developing hybrid DNA-lipid-based drug delivery vehicles directed towards biological membrane barriers, will hence likely emerge in the near-future.

Methods

Materials. 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N′-[4-(p-maleimidophenyl)butyramide] (MPB-DOPE) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N′-(cap biotinyl) (Biotin-DOPE) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). DOPC-Atto655 was acquired from Invitrogen (Ebersberg, Germany) and DiIC18(5) (DiD) from Thermo Fisher Scientific (Waltham, MA, USA). Single-stranded M13mp18 scaffold plasmid (p7294) was supplied by Bayou Biolabs (Metairie, LA, USA), as well prepared by Florian Praetorius using high-cell-density fermentation of Escherichia coli in stirred-tank bioreactors according to reference51. High purity salt free (HPSF) purified staple oligonucleotides for origami preparation, as well as 5′-Atto488, 5′-Alexa488 and 3′-Biotin-TEG-functionalized oligonucleotides (all HPLC-purified) were purchased from Eurofin Genomics (Ebersberg, Germany). 5′‘-Chol-TEG and 3′-Thiol-Modifier-C3 5′-functionalized oligonucleotides (all HPLC-purified) were acquired from Sigma-Aldrich (Taufkirchen, Germany).

Design and production of the DNA origami nanoscaffolds. The DNA origami structures employed throughout this work consisted in a 20-helix bundle with hexagonal lattice. As described in the main text, three curved designs were here

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**Fig. 5** Ultrastructure of lipid nanotubes decorated with DNA origami Q. a From confocal images, the membrane tubules obtained from GUVs (labeled with DOPC-Atto655; red) upon overnight incubation with structure Q3 (labeled with Atto488) appeared homogeneously covered with membrane-bound DNA origami. b Further cryo-EM imaging confirmed that the surface of the membrane tubules (black arrows) is densely covered with curved DNA nanostructures perpendicularly aligned along the long axis. c Based on the cryo-EM electron microscopy observations and radius of curvature of nanostructure Q3, a schematic representation of a lipid nanotube decorated with DNA origami Q is here depicted. Scale bars: (a) 5 μm; (b)100 nm.
developed origami $H$ (curvature $C = 21.7 \text{ nm}^{-1}$; curvature angle $\theta = 131^\circ$; radius of curvature $R = 46 \text{ nm}$; Supplementary Fig. 4; Supplementary Table 3), origami $Q$ ($C = 11.6 \text{ nm}^{-1}$; $R = 84 \text{ nm}$; Supplementary Fig. 3; Supplementary Table 1) and origami $L$ ($C = 0$; Supplementary Fig. 2; Supplementary Table 1). Those structures were based on the M13 p7249 plasmid and designed using CaDNAno52 (Supplementary Figs. 2–4). Initial 3D models (Supplementary Fig. 1) were predicted using CanDo55. Each design further included marked positions for attaching fluorophores, membrane-anchoring moieties or oligomerizing staples (Fig. 1d). More precisely, 7 sites on the bottom (concave) and top (convex) faces of the DNA origami (B0–B6 and T0–T6, respectively) plus 14 sites on the left and right facets (L0–L13 and R0–R13, respectively) were defined. This strategy allowed us to manipulate the functionality of the origami structures by exchanging the external positions with functionalized counterpart parts (Supplementary Notes), without compromising the shape of the nanostructures stabilized by the core staples. The edges of each of the 20 helical bundles, parts (Supplementary Notes), without compromising the shape of the nanostructures to manipulate the functionality of the origami structures by exchanging the right facets (L0–L13 and R0–R13, respectively) were de-

By using the Laser scanning confocal fluorescence microscopy, confocal imaging was performed on a commercial laser scanning microscope LSM 780 with a CoLoCoR unit (Zeiss, Jena, Germany) using a water immersion objective (C-Apochromat, 40 x/1.10, Zeiss, Jena, Germany). Atomic force microscopy (AFM) imaging was performed on a Philips CM100 transmission electron microscope operated at 100 kV17, 54. Images were recorded with an AmScope Ultra (JPK, Berlin, Germany) using the high-speed AC mode with USC-F03-k03 cantilevers (NanoWorld, Neuchâtel, Switzerland). The cantilever oscillation was turned to a frequency of 100–150 kHz, the amplitude kept below 10 nm. Scan rate was set to 5–25 Hz and setpoints close to 7–8 nm were utilized. Analysis of the AFM images was performed using JPK Data Processing (version 3.7.1.4). Negative-stain transmission electron microscopy (TEM) imaging was performed on a Philips CM100 transmission electron microscope operated at 100 kV17, 54. Images were recorded with an AmScope Ultra (JPK, Berlin, Germany) using the high-speed AC mode with USC-F03-k03 cantilevers (NanoWorld, Neuchâtel, Switzerland). The cantilever oscillation was turned to a frequency of 100–150 kHz, the amplitude kept below 10 nm. Scan rate was set to 5–25 Hz and setpoints close to 7–8 nm were utilized. Analysis of the AFM images was performed using JPK Data Processing (version 3.7.1.4). Neg
4 nM of origami Q0 or Q3 were pre-incubated for 30 min with DOPC MLV (at 0.5 mM lipid concentration) before deposition on the EM grids and negative staining. For cryo-electron microscopy (cryo-EM), 5 nM Q3 was pre-incubated overnight in a tube with DOPC GUVs. Samples were then adsorbed for 4 min on glow-discharged lacey carbon grids (Plano, Wetzlar, Germany) and vitrified by plunge freezing the grid in liquid ethane. Imaging was performed on a Titan Halo electron microscope (FEI, Eindhoven, Netherlands), equipped with a Falcon II camera and a Gatan 626 cryo holder (Pleasanton, CA, USA). The microscope was operated at 300 kV, with a magnification of x45,000, giving a pixel size of 0.237 nm at the specimen level. Data were collected using SerialEM at nominal –3.5 nm target defocus with an electron dose of 20 e⁻/Å². Tubular diameter (average ± s.d.) was obtained analyzing n = 35 parallel cross-sections along four Q3-decorated membrane tubules.

Estimation of the energetic costs for membrane bending. The energy required for membrane bending by curved DNA origami scaffolds Q and H and a BAR domain protein were calculated using the Area-difference Elasticity (ADE) model⁶⁶. This model, based on the classical Fehrenbacher-Ghosh classical elastic membrane model (spontaneous curvature model)⁶⁴, takes into consideration the finite thickness of the lipid bilayer and consequent additional penalty arising from the area difference between its two leaflets upon bending (i.e., negatively curved leaflet being compressed, while positively curved leaflet being expanded). The ADE model describes bending energy (εw) as:

$$\varepsilon_w = \frac{1}{2} \Delta A \left( C_1 + C_2 - \frac{4\pi}{\Delta A} \right)^{-1} \frac{\alpha}{2 \Delta \phi} \left( \Delta A - \Delta A_0 \right)^2$$

where $\Delta A$ is the bending modulus of DOPC bilayers (23.1 kJ/mol), $A$ is the area of the membrane segment, $C_1$ and $C_2$ are the principal curvatures (for a membrane tube, $C_i = 1/R$ and $C_0 = 0$), $\Delta A_0$ is the spontaneous curvature of the membrane, which relates to the intrinsic curvature of the lipid molecules. For a homogenous non-asymmetric bilayer, $C_0 = 0$. In the second term, $\Delta A$ is the differential monolayer area (determined by the difference in number of molecules of the outer and the inner monolayers) and $\Delta A_0$ is its value at equilibrium. $D$ corresponds to the membrane thickness. $\alpha = \pi/\kappa$, with $\kappa$ being the non-local bending rigidity modulus. $\alpha$ is estimated to be in the order of unity and the approximation $\alpha = 3/5$ was used.

Data availability. Data supporting the findings of this manuscript are available from the corresponding author upon reasonable request.

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Author contributions

P.S. and H.G.F. initially conceived the project. P.S. supervised and coordinated the project. H.G.F. supervised the development of curved DNA origami-based scaffolds. Experimental design was carried out largely by H.G.F., with contribution of A.K. H.G.F. designed the DNA origami structures, performed and analyzed the fluorescence microscopy experiments for studying requirements for membrane binding and curvature generation, executed the membrane deformation assays upon osmotic trigger and carried out the cryo-EM experiment. A.K. performed and analyzed the fluorescence imaging and FCS-based experiments for determining surface densities, binding and tubulation efficiencies. J.-P.S. developed the strategies for lateral self-assembly in solution and carried out negative-stain TEM experiments. All authors contributed to the writing and revision of the manuscript.

Additional information

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