Published in final edited form as:

Angew Chem Int Ed Engl. 2018 May 04; 57(19): 5330–5334. doi:10.1002/anie.201800141.

Vesicle tubulation with self-assembling DNA nanosprings

Michael W. Grome,

Department of Cell Biology & Nanobiology Institute, Yale University, 850 West Campus Drive, West Haven, Connecticut 06516 (USA)

Dr. Zhao Zhang,

Department of Cell Biology & Nanobiology Institute, Yale University, 850 West Campus Drive, West Haven, Connecticut 06516 (USA)

Dr. Frédéric Pincet, and

Laboratoire de Physique Statistique, Ecole Normale Supérieure, PSL Research University; Université Paris Diderot Sorbonne Paris Cité; Sorbonne Universités UPMC Univ Paris 06, CNRS, 24 rue Lhomond, Paris, 75005 (France)

Prof. Chenxiang Lin*

Department of Cell Biology & Nanobiology Institute, Yale University, 850 West Campus Drive, West Haven, Connecticut 06516 (USA)

Abstract

A major goal of nanotechnology and bioengineering is to build artificial nanomachines capable of generating specific membrane curvatures on demand. Inspired by natural membrane-deforming proteins, here we design DNA-origami curls that polymerize into nanosprings and show their efficacy in vesicle deformation. DNA-coated membrane tubules emerge from spherical vesicles when DNA-origami polymerization or high membrane-surface coverage occurs. Unlike many previous methods, the DNA self-assembly-mediated membrane tubulation eliminates the need for detergent or top-down manipulation. The DNA-origami design and deformation conditions have substantial influence on the tubulation efficiency and tube morphology, underscoring the intricate interplay between lipid bilayers and vesicle-deforming DNA structures.

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Spring forward: ESCRT-inspired DNA-origami curls equipped with amphipathic anchors draw tubules from vesicles when the curls polymerize on membrane into nanosprings. Alternatively, vesicle tubulation occurs at sufficiently high surface concentration of DNA curls, independent of DNA-origami polymerization. The deformation outcome can be modulated by the DNA-origami design and experimental conditions.

* chenxiang.lin@yale.edu.

Keywords

DNA nanotechnology; DNA origami; Membrane deformation; Vesicles; Self-assembly

Complex and dynamic membrane shapes are hallmarks of living cells.^[1] Tubular shape is one of the most common membrane architectures that exists during cellular events including endocytosis, viral budding, and cytokinesis. Various membrane-deforming proteins that sense and generate membrane curvatures contribute to the formation and stabilization of membrane tubules in cells.^[2] While many existing techniques can recapitulate membrane tubulation *in vitro*,^[3] experimental restraints (e.g., dependence on lipid composition, detergents, or mechanical force) pose limitations on a method's programmability and applications.

Stemming from the simple idea of combining branched DNA motif with complementary “sticky-ends”,^[4] the field of structural DNA nanotechnology has matured into a stage where one can easily generate three-dimensional nanostructures with programmable geometry, surface chemistry, and dynamics.^[5] In recent years, the field has seen considerable progress towards using DNA nanostructures for membrane engineering in order to better control synthetic membrane properties as well as to manipulate biological membranes.^[6] For example, supported lipid-bilayer membranes have been coated with DNA “rafts” and promoted DNA-tile association;^[7] DNA nanochannels labeled with hydrophobic moieties have been developed for membrane penetration;^[8] DNA templates have been engineered to guide the assembly of size- and shape-defined liposomes and to induce membrane fusion and bending.^[3e, 9] Particularly relevant to this work, the lateral association of cholesterol-modified DNA nanoblocks on membrane have led to vesicle flattening, presumably imposed by a large flat DNA surface.^[10] Although inserting DNA nanochannels at high concentration can result in tubule-like structures on giant unilamellar vesicles (GUVs), such deformation is likely driven by surface crowding; the exact mechanism remains unclear.^[8b] Therefore, engineering DNA nanostructures capable of programmable vesicle tubulation remains challenging.

While existing membrane-deforming DNA structures mostly mimic BAR-family proteins,^[10] we took our design inspiration from dynamins^[2a] and ESCRT machineries^[2c], two major classes of proteins that polymerize into helical structures coating lipid tubules. Specifically, we set out to generate DNA curls that would self-assemble into helical structures similar to the Snf7 filament, which form spiral-like assemblies on membrane with outer diameter of 50–100 nm and filament thickness of 5–10 nm.^[11] To achieve this, we bent and twisted a ~100-nm long, 14-nm thick DNA 24-helix-bundle rod a –55° of bend per 77-bp was achieved by inserting and deleting equal number of base-pairs on the opposite sides of the DNA rod^[12] and the twist was implemented by changing the bending axis by 30° every 77-bp. The result of such a design is a ‘C’-shaped structure with an out-of-plane twist (Fig. 1a, S1, and S2). To render the DNA structure with stronger membrane affinity, we reserved 24 single-stranded DNA (ssDNA) extensions along the inner surface of the DNA-origami curl for attaching amphipathic peptides as membrane anchors, which we designed to mimic the N-terminal ANCHR helix of Snf7.^[13] The distance between neighboring peptides

is 4–7 nm, close to their ~3 nm spacing in snf7 filaments.^[14] Gratifyingly, upon conjugation with Cy5-labeled ssDNA, this peptide dissolved readily in detergent-free aqueous buffer solutions, bound well to the attachment sites on the DNA curl, and had minimal tendency to aggregate DNA nanostructures (Fig. 1b and S3). We further designed a set of “linker strands” that would bridge the front and rear ends of the DNA curls, causing monomeric DNA curls to polymerize and form “nanosprings” with an expected inner diameter of 27 nm and a helical pitch of 53 nm. Indeed, adding linkers to the peptide-labeled DNA curls triggered polymerization; when examined by negative-stain transmission electron microscopy (TEM), the resulting nanosprings measured a length of 330 ± 190 nm with an inner diameter of 26 ± 5 nm and a helical rise of 80 ± 9 nm (Fig. 1c). The discrepancy between designed and measured nanospring dimensions is likely due to the structural distortion on TEM grids^[15] and the overwound DNA caused by numerically balanced insertions and deletions^[16]. A considerable amount of closed DNA circles also emerged after addition of linkers as a result of self-closing dimers. It is worth noting that the DNA-curl polymerization is tunable by changing Mg^{2+} and K^+ concentrations. The polymerization in solution is optimal at 10 mM Mg^{2+} without K^+ ; higher Mg^{2+} promoted nanospring aggregation and higher K^+ led to shorter polymers (Fig. S4).

After establishing the folding of DNA curls and their polymerization in solution, we then tested their ability to bind and deform vesicles. The experimental procedure is summarized in Fig. 2a. Briefly, purified DNA-curl monomers (4 nM) labeled by ANCHR-mimicking peptide were incubated with extruded liposomes (99.2 μ M 1,2-dioleoyl-sn-glycero-3-phosphocholine or DOPC, 0.8 μ M 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl); mean diameter: 134 nm) at 4°C for 1 hour to allow for binding before mixed with linker strands and further incubated at 30°C for up to 32 hours; the osmolarity of the solution was kept constant throughout the entire process. TEM analyses (Fig. 2b) on the mixture of DNA curls and liposomes showed their binding depended on the presence of membrane anchors. To better analyze the liposome binding affinity, we subjected the mixture of DNA-curl monomers and liposomes to isopycnic centrifugation. A fluorescence scan of the recovered fractions confirmed the membrane-anchor dependent binding between Cy5-labeled DNA curls and rhodamine-labeled vesicles (Fig. 2c), which co-migrated with amphipathic peptides decorating DNA curls but parted ways in the absence of these peptides. However, in addition to DNA-curl-bound liposomes, TEM revealed free DNA curls and naked liposomes in the fractions where the two coexisted (Fig. S5), suggesting their binding is transient. Interestingly, individual ANCHR-mimicking-peptide-DNA conjugate molecules showed no detectable affinity towards vesicles (Fig. S5), highlighting the DNA curl’s role in facilitating membrane binding, possibly by both bringing together multiple (a maximum of 24) membrane anchors and providing a negatively charged surface to interact with the zwitterionic lipid head group in the presence of magnesium cations. Adding linker strands after the initial binding step resulted in the formation of membrane tubules wrapped by DNA nanosprings (Fig. 2d and S6). Among the deformed liposomes, some retained a spherical body with a tubule protruding outwards while others turned entirely into a tubular shape. A time-course study showed that the tubule abundance and length reached maximum after the first two hours of polymerization and stayed stable for 16 hours; another 16-hour incubation at 30°C led to fewer and shorter

tubules, likely because of degradation (Fig. 2e and S6). We note that vesicle tubulation occurred in a solution with 10 mM Mg^{2+} and 100–800 mM K^+ , despite the fact that the DNA-curl polymerization in solution was most efficient without K^+ . This is because monovalent cations weaken the electrostatic interactions between DNA and lipid, hence mitigating DNA-curl induced vesicle aggregation (Fig. S7 and S8). Neither polymerizing membrane-anchor-free DNA curls in the presence of vesicles (Fig. S9) nor incubating pre-polymerized membrane-anchor-labeled DNA nanosprings with vesicles (Fig. S10) could generate membrane tubules, indicating that vesicle tubulation necessitates membrane-binding DNA-curl monomers. Despite the low deformation yield (<4% of vesicles were deformed after 16 hours), the membrane tubules unambiguously formed as a result of DNA-nanostructure coating. In fact, nuclease digestion of the DNA coat after tubulation resulted in the disappearance of the vast majority of membrane protrusions and tubular vesicles (Fig. S11).

A closer inspection of the apparent membrane tubule width and the associated nanospring periodicity identified two types of tubes (Fig. 3a). The first type features a tubule diameter of 15 ± 2 nm and an apparent helical pitch of 35 ± 6 nm (peak-to-peak distance of the DNA coat). The second type of tubule is considerably wider (diameter: 29 ± 4 nm) with a more densely packed DNA-curl coating (apparent pitch: 16 ± 2 nm). In rare cases, a continuous membrane tubule exhibits both morphologies in different segments. One possible explanation is that the wider tubes resulted from the dehydration and flattening of the thinner tubes on TEM grids. An alternative hypothesis is that both types of membrane tubules existed in solution and the wider ones formed because of higher membrane coverage by DNA curls. To test this hypothesis, we increased the concentration of DNA-curl monomers from 4 nM to a maximum of 20 nM, which translates to an increase of the maximal vesicle-surface coverage from 16% to 80%. The higher surface coverage led to two significant changes (Fig. 3b, 3c, and S12). First, the overall deformation yield (the portion of vesicles that undergo notable tubulation) increased with a higher initial concentration of DNA-curl monomers, up to ~13% with 20 nM DNA curls. Second, the occurrence of wider tubules increased with greater membrane coverage and dominated the tubule population with DNA-curl concentration greater than 10 nM (or roughly 40% maximal surface coverage). Reducing the DNA curl and lipid concentration proportionally resulted in a similar trend, confirming that surface coverage is the main determinant of deformation efficiency and tubule morphology (Fig. 3c and S13). Based on these data, our interpretation is that the wider tubules' diameters are likely defined by the multiple, moderately compressed DNA springs, whereas the narrower tubes, surrounded by only 1–2 slightly compressed springs are free to adjust their diameters to approach the minimum allowed by the membrane bending energy (see Technical Notes in SI for more discussion). The compressed DNA springs have to adhere to membrane with sufficient strength (through both electrostatic and hydrophobic interactions) to prevent the retraction of lipid tubules, which exposes membrane anchors. Intriguingly, an appreciable amount of tubules (width: 24 ± 8 nm, max. yield ~5%) was produced at high membrane coverage conditions (Fig. 3d and S14) without linker strands, where the tubules were covered by DNA curls, but with less regular patterns. This linker-independent tubulation suggests a different, although less efficient, deformation mechanism driven by surface

crowding and membrane-anchor insertion, consistent with the notion of coupling between protein/lipid shape and membrane curvature.^[1, 17]

Having confirmed the DNA nanospring's capability in deforming organelle-size large unilamellar vesicles (LUVs), we attempted to test their tubulation efficacy on GUVs with sizes approximating eukaryotic cells. We first incubated Cy5-labeled DNA-origami curls with nitrobenzoxadiazole (NBD)-labeled GUVs (lipid concentration $\approx 10 \mu\text{M}$) at 4°C for 1 hour in an iso-osmotic buffer solution containing 10 mM Mg^{2+} and 100 mM K^+ . We then held this mixture (with or without additional linker strands) at 30°C for 16 hours and monitored vesicles using a confocal fluorescence microscope. The DNA curls bound to GUVs (Fig. 4a) in a similar way as they bound to LUVs: the surface coverage of DNA curls depended on their initial concentration and membrane-anchor density (Fig. S15); the bound and unbound DNA curls were in a dynamic equilibrium with slow off-rate (Fig. S16). With 20 nM DNA curls, noticeable tubulation occurred after at least 1.5 hours of incubation at 30°C . After 16 hours, these outward protrusions grew long (some longer than $250 \mu\text{m}$) and abundant. The tube-like structures carried both Cy5 and NBD fluorescence (Fig. 4a), suggesting they were coated by DNA curls, as confirmed by negative-stain TEM studies. Under TEM, a deformed GUV usually featured multiple emanating protrusions (Fig. 4b). Comparing to the membrane tubules originated from LUVs, these GUV protrusions were much wider ($116 \pm 50 \text{ nm}$) with extremely dense DNA coating. The DNA curls lay closely against one another in parallel at the edges of the protrusions but showed no apparent order in the central areas, albeit the high surface density (Fig. 4c). We found that the linker strands, but not the membrane-binding DNA curls, were dispensable for tubulation (Fig. S17); the vesicles were deformed rather similarly with or without linkers (Fig. S18 and S19). Further, DNA curls with cholesterol modification and DNA rings (outer diameter: 60 nm) with ANCHR-mimicking peptide modification on the inside deformed vesicles (GUVs and LUVs) in a similar manner and without linker induced DNA-origami oligomerization (Fig. S20–S23). Changing peptide anchors to cholesterol increased the aggregation of DNA curls, but did not reduce their tubulation capability in general. Although the influence of membrane-anchor type and DNA nanostructure geometry on vesicle deformation warrants further study, the phenomena observed here, combined with the linker-independent LUV deformation, strongly support that DNA-origami structures can drive vesicle tubulation solely by introducing a large number of membrane anchors and covering the vesicle surface with DNA structures at high density.

In summary, we have built bioinspired nanostructures that drive tubular deformations from spherical vesicles. Such a membrane-sculpting structure comprises three modules: a structural module (the curved DNA-origami body) that provides a negatively charged interior with dozens of “open seats” (ssDNA extensions) for guest molecules, a membrane-anchoring module (DNA-peptide or DNA-cholesterol conjugate) that decorates the DNA curl's inner surface and confers hydrophobicity, and a connector module (linker strands) that join the ends of monomeric structures to form a long helical filament. Both membrane incorporation and DNA-origami polymerization provide energy to overcome membrane tension and intrinsic curvature for vesicle deformation. Embedding amphipathic anchors into lipid bilayers expands the outer leaflet and introduces higher membrane curvature; the elongation of DNA springs “pulls” on the lipid and “holds” the shape of the membrane

tubules (see Technical Notes in SI for more discussion). The vesicle deformation takes two different forms that both require the membrane-anchoring module and DNA structures surrounding the membrane tubules, but result in distinct tubule morphology and DNA-coating patterns. This process can be modulated by changing the surface density of DNA nanostructures (systematically tested) and the membrane tension of vesicles (uncontrolled in this study). With sparse DNA coating and high membrane tension, the connector module is essential for making tubules wrapped by nanosprings. In contrast, when a low-tension membrane surface is saturated with membrane-anchor-labeled DNA structures, low energy cost, polymerization-independent tubulation becomes favorable. This may explain the dispensable linker strands in GUV deformation, where membrane tension is likely lowered due to evaporation and the DNA curls are in ~8-fold excess for complete membrane coverage. Additionally, higher surface coverage and lower membrane tension can both increase tubulation efficiency. Tension-dependent vesicle tubulation has been demonstrated using motor proteins^[3c] and concentration-dependent self-assembly on membrane has been observed with curvature sensing/inducing proteins (e.g., amphiphysin^[17b] and FtsZ^[18]). Deforming membranes with engineered synthetic constructs in a way that resembles the work of naturally existing proteins is not only a major feat in synthetic biology,^[19] but also provides a tool to study the membrane remodeling mechanisms. The modular self-assembling nanosprings extend our membrane manipulating capability and pave the way for future development of more sophisticated, multistep membrane-sculpting machineries (e.g., with pre-inhibited components that are activated upon membrane binding) that can sort cargo molecules, drive vesicle budding, alter deformation topology (i.e., tubulation toward vesicle lumen), and act on cell membranes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank C.G. Burd for discussion and designing ANCHR-mimicking peptide. This work is supported by a National Institutes of Health (NIH) Director's New Innovator Award (DP2-GM114830), an NIH grant (R21-GM109466), and a Yale University faculty startup fund to C.L. and a European Research Council (ERC) funded grant under the European Union's Horizon 2020 research and innovation programme (grant agreement no. 669612) to F.P. In addition, M.W.G. is supported by an NIH training grant (T32-GM007499).

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Figure 1.

The design and in-solution assembly of DNA-origami nanosprings. (a) Main panel: Cartoon models illustrating the design of a 24-helix-bundle DNA curl (bent grey cylinders) labeled with up to 24 amphipathic membrane-binding molecules (purple spheres) and the linker-strand triggered polymerization of such curls into a left-handed DNA nanospring. Inset: cartoon model and sequence of the DNA-peptide conjugate that decorates the interior of the DNA curl (underlined letters: hydrophobic amino acids). (b) Assembly results of the peptide-labeled DNA-curl monomers. Left: A representative negative-stain TEM image of the purified monomeric curls. Right: An agarose gel resolving DNA curls with and without

ssDNA extensions (handles) in the presence and absence of Cy5-modified DNA-peptide conjugates. Pseudo-color fluorescence: red= Cy5, green=ethidium bromide. (c) A representative TEM image of DNA nanosprings. Scale bars: 100 nm

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Figure 2.

DNA-nanospring induced tubulation of LUVs. (a) A schematic drawing of the two-step deformation experiment. (b) Representative TEM images of rhodamine-labeled DOPC LUVs after incubation with DNA curls carrying Cy5-labeled DNA-peptide conjugate (left) and Cy5-labeled ssDNA only (right). (c) The mixtures shown in (b) after isopycnic centrifugation. Pseudo-color fluorescence of recovered fractions: red=Cy5, blue=rhodamine. Smaller numbers denote lighter fractions. (d) Selected TEM images of deformed vesicles after the polymerization step. (e) Deformation efficiency and (f) membrane tubule length as a function of polymerization time. Error bars represent standard deviations. For each

deformation experiment, 1400–2000 vesicles were examined (e) and 17–23 tubules were measured (f). DNA curl and lipid concentrations were 4 nM and 100 μ M, respectively. Scale bars: 100 nm.

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Figure 3.

The impact of vesicle-surface coverage on LUV tubulation. (a) Two types of membrane tubule morphologies under negative-stain TEM. A cartoon model (yellow: membrane tubule, grey: DNA nanosprings) is placed over representative images. (b) Membrane tubule morphologies (characterized by width vs apparent nanospring pitch) resulting from six different lipid and DNA-curl concentrations. Red and blue circles highlight the wide and narrow tube populations, respectively. For each scatter plot, 20–27 tubules were analyzed by TEM. (c) Tubule abundance and morphology as a function of vesicle-surface coverage by DNA curls at two different lipid concentrations. For each of the six bars, 800–4200 vesicles were examined by TEM. The ratio between wide (red) and narrow (blue) tubes was extracted from (b). (d) Linker-independent tubulation. A cartoon model is placed over representative negative-stain TEM images. For all experiments, DOPC vesicles were incubated with amphipathic-peptide-labeled DNA curls first at 4°C for 1 hour and then at

30°C for 16 hours; linker strands, if used, were added after the first hour. Scale bars: 100 nm.

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Figure 4.

DNA-curl induced GUV deformation. (a) Representative fluorescence microscope images showing the NBD-labeled DOPC GUVs binding with (left) and deformed by (right) Cy5-labeled DNA curls carrying amphipathic peptides in the absence of linker strands. Pseudo-color fluorescence: red=cy5, green=NBD. Scale bars: 25 μm . (b) A representative negative-stain TEM image showing a deformed GUV. Scale bar: 200 nm. (c) Close-up views of selected areas of deformed vesicles under negative-stain TEM. A cartoon model (yellow: membrane tubule, grey: DNA curls) is placed to the right of the corresponding image. The tube in the left image is attached to a GUV (not shown in the picture). Scale bars: 200 nm.