

DNA Nanostructures on Membranes as Tools for Synthetic Biology

Aleksander Czogalla,^{1,*} Henri G. Franquelim,² and Petra Schille^{2,*}

¹Department of Cytochemistry, Faculty of Biotechnology, University of Wrocław, Wrocław, Poland; and ²Department of Cellular and Molecular Biophysics, Max Planck Institute of Biochemistry, Martinsried, Germany

ABSTRACT Over the last decade, functionally designed DNA nanostructures applied to lipid membranes prompted important achievements in the fields of biophysics and synthetic biology. Taking advantage of the universal rules for self-assembly of complementary oligonucleotides, DNA has proven to be an extremely versatile biocompatible building material on the nanoscale. The possibility to chemically integrate functional groups into oligonucleotides, most notably with lipophilic anchors, enabled a widespread usage of DNA as a viable alternative to proteins with respect to functional activity on membranes. As described throughout this review, hybrid DNA-lipid nanostructures can mediate events such as vesicle docking and fusion, or selective partitioning of molecules into phase-separated membranes. Moreover, the major benefit of DNA structural constructs, such as DNA tiles and DNA origami, is the reproducibility and simplicity of their design. DNA nanotechnology can produce functional structures with subnanometer precision and allow for a tight control over their biochemical functionality, e.g., interaction partners. DNA-based membrane nanopores and origami structures able to assemble into two-dimensional networks on top of lipid bilayers are recent examples of the manifold of complex devices that can be achieved. In this review, we will shortly present some of the potentially most relevant avenues and accomplishments of membrane-anchored DNA nanostructures for investigating, engineering, and mimicking lipid membrane-related biophysical processes.

Synthetic biology is a relatively new discipline that combines biological insights and aims with quantitative sciences and engineering expertise. One of its fundamental variants is bottom-up synthetic biology, based on the concept of building artificial biological systems with a minimal set of functional elements *de novo* (1). Compared with the more biotechnology-inspired reengineering of living cells, this approach is less directed toward production and application, and more toward fundamental understanding of biological systems. Although the ultimate goal of bottom-up synthetic biology is to create a minimal living cell, it serves also as a versatile approach to characterize interactions between biomolecules under well-defined conditions, usually impossible to achieve in complex biosystems. Concise quantitative information obtained by the bottom-up approach helps to elucidate the minimal set of structural and functional modules required to reconstitute specific cellular phenomena. Of importance, these principle motifs and modules of functionality do not need to be restricted to original cellular elements,

as other biocompatible synthetic molecules and biologically inspired systems could perform analogous functions in a similar fashion, often with higher efficiency and robustness. Moreover, such molecules may provide features that allow better external control.

In nature, most of the cellular processes are mediated by proteins. On the other hand, structural organization and compartmentalization within a cell typically involves lipid membranes as functional interfacial barriers. Peripheral and integral membrane proteins participate in various fundamental phenomena, like energy conversion, signal transduction, intracellular transport, and communication, to name a few. Thus, the relation between structure and function of these proteins and the features of lipid membranes is of particular interest. However, due to their amphipathic character, such membrane-active molecules are usually difficult to obtain in sufficient amounts and purity, and the risk of compromising their native state while reconstituting them in artificial membrane systems is relatively high (2). This makes it desirable to find innovative experimental approaches based on the idea of synthetic functional analogs of membrane proteins. Despite the different chemical nature of DNA compared to proteins, its high binding fidelity, sequence specificity, directionality of hybridization, and ability to form asymmetric interactions with its complement

Submitted November 4, 2015, and accepted for publication March 7, 2016.

*Correspondence: aleksander.czogalla@uwr.edu.pl or schille@biochem.mpg.de

Editor: Brian Salzberg.

<http://dx.doi.org/10.1016/j.bpj.2016.03.015>

© 2016 Biophysical Society

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

triggered the use of DNA-lipid hybrid assemblies as versatile tools to mimic membrane processes (3). Most recently, the DNA origami approach (4) opened up exciting new perspectives in this field. Now it is possible to put more effort on rational design and efficient fabrication of molecules mimicking structural and functional features of proteins, without the risk of low yield or instability. Although artificial, these systems are fully biocompatible and already proved their usefulness in studying physicochemical features of membrane assemblies, which are important for a range of applications in basic biological research, medicine, and nanotechnology.

Complex biological phenomena such as membrane transformation, membrane fusion, endocytosis, signaling, secretion, or ion conductance could now be studied and reconstituted in a minimalistic manner on model membranes. In this review, we will thus focus on the innovative engineering capabilities of DNA as a synthetic biology tool, to study lipid membranes biophysics and mimic essential biological functions performed by membrane proteins.

Variety of DNA structures for synthetic biology

DNA seems to be a perfect molecule for synthetic biology approaches. Sequence-specific and directional hybridization of DNA duplexes occurs with high fidelity and the binding stability is affected by several controllable parameters, such as temperature, ionic strength, and DNA concentration. On the other hand, the complexity of its sequence is significantly reduced when comparing to proteins, which makes design procedures easier. DNA, which is chemically and biologically more stable than RNA, is widely used in biophysical studies and can be easily synthesized in a lipophilic form. Conjugation of one or several lipophilic (lipid-like) moieties with a nucleic acid, typically via neutral linkers (e.g., polyethylene glycol), yields lipophilic nucleic acids. Such molecules allow combining specific recognition features of the highly water-soluble nucleic acid strand with controllable functionalization of cell membranes and lipid vesicles. Features such as self-assembly, membrane incorporation, and affinity for specific nucleic acid recognizing molecules can be realized by independently modulating anchor, linker, and nucleic acid. Amphipathic oligonucleotides have been heavily exploited to mimic membrane association processes usually mediated by proteins in natural systems. On the other hand, larger DNA nanostructures enabled to introduce multifunctionality and structural aspects into synthetic biology. The DNA origami concept, i.e., arranging nucleic acid molecules into two-dimensional (2D) and three-dimensional (3D) custom-shaped nanometer-scale objects, led to a major breakthrough in the field of nanotechnology (4,5). Here, a large number of short oligonucleotides (staples) bind to defined segments of a long single-stranded DNA scaffold molecule in a sequence-specific manner (6). Individual staple strands can hybridize with multiple scaffold-strand segments,

which results in constraining of the latter to double-helical structures in a defined array. Introduction of insertions and deletions of base pairs within staples enable twisting and curving of the nanostructures at specific angles (7). Moreover, origami DNA nanostructures can be hierarchically assembled via staple strands that couple several scaffold strands together (4). Another unique feature of this approach is the possibility to functionalize individual staple strands with chemically modified groups, which allows nanometer-precise positioning of structural and functional elements (e.g., linkers, fluorescent dyes, proteins, nanoparticles, etc.) within the molecules (8). Functionalization with amphipathic moieties enables DNA origami nanostructures to bind to lipid membranes. Selective functionalization is also applicable in the DNA tile self-assembly approach (9), which is based on the hybridization of many short DNA subunits consisting of a few interconnected oligonucleotide segments (<5 sequences). Several categories of DNA tiles have been reported, from rigid multiple crossover molecules with sticky ends for lateral oligomerization (10) to multidomain single-stranded sequences able to form 2D (11) or 3D brick-like building blocks (12). With these techniques in hand, it is possible to create molecules of virtually any shape having a desired pattern of various functional moieties.

Taking all these features into consideration, in the following sections we will present in detail the different levels of complexity associated with the various membrane-active lipophilic modules that can be engineered using DNA nanotechnology.

Lipid anchors in DNA nanotechnology

Because the synthesis of DNA conjugates requires chemical expertise, commercially available nucleotides modified with triethyleneglycol-cholesteryl (TEG-cholesteryl) (Fig. 1 A) at either 5' or 3' became most popular. Porphyrin is extraordinary due to its multifunctionality, as it can also participate in transfer of energy or electrons and coordination of ligands (13). Cholesterol and tocopherol anchors let the modified nucleic acids insert quickly into model lipid membranes, and the insertion is reversible (14,15). It should be noted that cholesteryl-TEG anchors, whereas mixed 1/4 mol/mol with phospholipids, do not disturb the bilayer structure and dynamics of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) membranes and do not induce condensation of the membrane lipids, in contrast to cholesterol (16). Individual cholesteryl-anchored single-stranded oligonucleotides insert into supported lipid bilayers or lipid vesicles as monomers, and their conformation switches to more rigid (transition from mushroom to brush state) as the concentration increases, which also influences hybridization kinetics with complementary oligonucleotides from the solution (17–19). In the case of multiple cholesteryl counterparts, bilayer insertion is much more dependent on the self-assembly state in solution. Indeed, the amphipathic

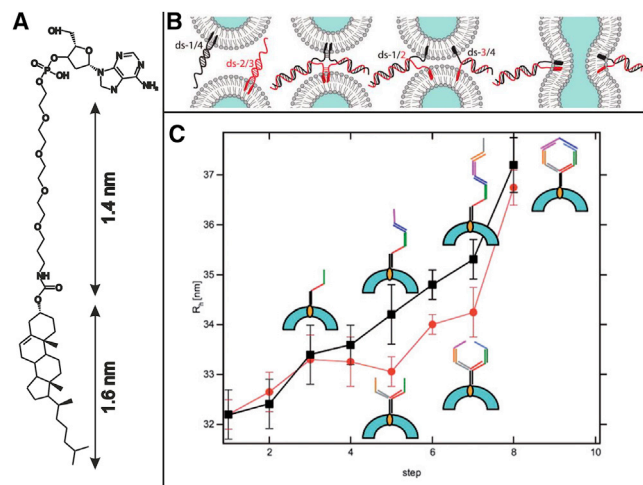


FIGURE 1 Lipophilic nucleic acids and their assembly on liposomes. (A) Cholesteryl-TEG-nucleotide. (B) Schematic representation of vesicle fusion induced by cholesteryl-TEG-oligonucleotide zippers in analogy to SNARE Fusion complex (reproduced with permission from (43), copyright 2008 American Chemical Society). (C) Hydrodynamic radii (R_H) of liposomes during step-by-step assembly of DNA pseudo-hexagons on their surface (squares: asymmetric protocol, circles: symmetric protocol) (reproduced with permission from (21), copyright The Royal Society of Chemistry). To see this figure in color, go online.

character of these molecules causes their aggregation in aqueous solution with the onset at $10 \mu\text{M}$ for the single cholesteryl conjugates and at $0.2 \mu\text{M}$ for the multiple cholesteryl derivatives (18). Single cholesterol anchors appear to be sufficient to functionalize liposomes with 38-base DNA-aptamers for cell-specific targeting (20) and membrane-assisted assembly of DNA structures (21). Double cholesteryl anchoring of oligonucleotides results in enhanced hydrophobic association with membranes (14,22); however, as some of the cholesterol anchors do not insert into the membrane, aggregation at the interface may occur (17,18). Although sterol-mediated anchoring has been the prime choice for binding DNA to membranes, alkyl chains have been recurrently used as membrane anchors of nucleic acids, too (23,24). Regarding the functionalization of oligonucleotides with diacyl lipid moieties, phosphoramidite- or maleimide-lipid headgroup modifications have been widely employed to covalently attach desired DNA sequences on diacylglycerol-(phospho)lipids (23,25). Double palmitoylated peptide nucleic acids (PNA) have been also synthesized for this purpose (24). Alternative membrane anchoring strategies not involving lipophilic moieties are further viable, and streptavidin-mediated anchoring of biotinylated oligonucleotides to biotin-functionalized lipid headgroups being inarguably the most valid possibility (26).

Functional membrane-anchored oligonucleotides

The nucleic acid moieties of membrane-anchored cholesteryl-functionalized oligonucleotides are free to bind their

complementary molecules from the aqueous environment in a reversible way. Remarkably, when compared to the oligonucleotide hybridization kinetic rate in solution, at low membrane grafting densities the rate constant of hybridization is faster, although at higher densities it is slower (19). Even more interesting is to use such principles for controlling processes of higher complexity, as in the case of the cholesteryl-oligonucleotide-mediated complementary interactions of two populations of liposomes of various sizes (3). Studying assembly processes of different membranous compartments is not only a way to extrapolate to natural systems with their complex intracellular vesicular machinery (i.e., endoplasmic reticulum-Golgi-plasma membrane trafficking), but also contributes to the concept of microdevices for the controlled confinement, transport, and manipulation of vesicular cargo. Here, DNA hybridization could be employed to pinpoint essential physical mechanisms underlying vesicle-vesicle interactions. Apparently, as the lipid-anchored DNA is free to diffuse in the plane of a membrane, adhesion plaques between pairs of liposomes saturate at ~ 20 DNA molecules per vesicle. Above this threshold, excess DNA can bind additional liposomes and ultimately large liposomal precipitates can form. In the case of higher numbers of complementary membrane-anchored DNA pairs, formation of multicompartments made of at least three different liposomes can be achieved (26). With such an approach, multilayer aggregates could be created, where each layer is composed of a unique population of membrane-embedded aqueous compartments (27), which can be potentially used in organization and regulation of biochemical reactions on a nanoscale. Reversible cross-linking rather than clustering of lipid vesicles is possible with DNA strands modified by hydrophobic anchors at both ends (28). Here, the single-stranded DNA molecule inserts both lipophilic anchors into the same vesicle, but upon hybridization with unmodified antisense DNA strands, the rigidity of the double helix increases, and one of the membrane anchors is thus released into solution allowing interliposomal anchoring to occur. Such reversible assembly of liposomes provides a precise switch between assembled and disassembled states of multicompartimentalized membranous systems.

Membrane-anchored DNA oligonucleotide hybridization also allows investigating phenomena associated with membrane-surface tethering and ligand-receptor docking. The self-sorting of tethered membranes, via lipophilic DNA anchors, on top of supported lipid bilayers has interesting biological implications for modeling lateral domain organization of membrane surface receptors and cell-to-cell junctions (29). Here, the laterally mobile lipid-DNA conjugates are prone to segregate by their height (8–24 nm hybrids) and the upper tethered lipid bilayer can deform to accommodate this height difference. A similar approach was used to follow the lateral docking dynamics of two populations of vesicles attached via lipophilic

oligonucleotides to a supported lipid bilayer (30). Such a strategy provides a simplified system that mimics cellular membrane-membrane recognition and 2D-binding events. It is worth mentioning that DNA-mediated tethering of lipid vesicles on surfaces, either at fixed points or on supported lipid bilayers, is a broadly used method to lower the dimensionality of a studied system, which helps to monitor membrane-related events with sensitive surface techniques, including fluorescence recovery after photobleaching (31), total internal reflection fluorescence microscopy (32), quartz crystal microbalance, and surface plasmon resonance (33). Supported lipid bilayer-mediated assembly of liposomes seems therefore to be a promising approach in creating and mimicking complex multicompartments assemblies.

When investigating the binding to lipid membranes of charged molecules such as oligonucleotides, a key factor to consider is the effect of lipid composition itself. For example, thermal stability of DNA duplex formation in vesicle aggregates is notably higher when compared to the free DNA sequences, nevertheless, this property strongly depends on the lipid charge of the vesicles membranes (34). However, this is only the case for relatively short (10 base) cholesteryl-DNA conjugates, where anionic 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) membranes lower the melting temperature of vesicle agglomerates and cationic 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) increases it, when compared to liposomes composed of neutral POPC (34). Besides charge, the phase properties of lipid bilayer membranes are also of major importance, because they are postulated to contribute to their physiological functions (35). Membranes of multicomponent lipid composition can be patterned by phase separation into distinct phase states, including liquid-disordered (Ld), liquid-ordered (Lo), and solid-ordered gel, where the latter is characteristic for lipids with saturated acyl chains in the absence of cholesterol. For model systems, the generally accepted view is that the majority of membrane-associated proteins will partition into less tightly packed Ld states, with the exception of proteins that have a specific tag with a strong affinity to Lo domains or to the border of two phases (36,37). Similarly, membrane-anchored oligonucleotides will partition between coexisting phases, which may result in the appearance of domains with distinct functionalities in a membrane. Such behavior was reported for DNA strands modified by two tocopherol membrane anchors (15,38), which on giant unilamellar vesicles (GUVs) composed of POPC/sphingomyelin/cholesterol (molar ratio 1:1:1) were localized in the Ld domains. Cholesteryl-modified oligonucleotides, on the other hand, behave differently on the surface of Lo/Ld phase-separated vesicles. Here, single-anchored DNA partitioned almost evenly between coexisting phases of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)/1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine/cholesterol GUVs, but Lo domains were

preferably decorated by doubly-anchored cholesteryl-DNA (22). The shift toward Lo partitioning can be enhanced by incorporation of highly unsaturated lipids with small headgroups, like bovine heart cardiolipin, which increases lateral packing stress within the Ld phase (39). Similar to double-cholesterol-anchored DNA, oligonucleotides with two unsaturated C18 alkyls partition preferentially into Lo domains of phase-separated vesicles (39). When populations of such vesicles functionalized by DNA strands of complementary sequences were combined, size-limited multivesicle aggregates were observed, in which the Lo domains were saturated within the adhesion plaques. Even more interesting is a switchable system employing a double tocopherol-conjugated DNA and double palmitoylated PNA of opposite charge (40). These molecules located preferentially to Ld and Lo phases of individual GUVs, respectively. Addition of oligonucleotides that hybridize with both membrane-anchored nucleic acids caused partitioning of the resulting complex into Ld domains, which could be reversed by treatment with nuclease (Fig. 2 A).

In biological systems, besides selective recognition and docking of membrane-confined compartments, one of the dynamic aspects of major relevance is to controllably release or generally exchange the content of such compartments. Those processes can be mimicked using lipid vesicles and amphipathic DNA. An illustrative example is selective cargo release of liposomes functionalized with DNA block copolymers (41). Here, stable membrane-anchored DNA protruding handles can be hybridized with oligonucleotides linked to a photosensitizer. Light irradiation then results in local lipid oxidation and, as a consequence, loss of membrane integrity. Cargo release depends on the DNA sequence code on the surface of the vesicles.

One step further toward a synthetic biological system is the use of lipid-DNA conjugates mimicking the SNARE complexes, which makes it possible to systematically fine-tune biochemical features and observe how this affects the rate and efficiency of membrane fusion events. Employing this idea resulted in creation of artificial secretory cells composed of minimal exocytic machinery (42). In this approach, GUVs filled with catechol-loaded small liposomes were mimicking plasma membrane of the cell and intracellular vesicles, respectively. The SNARE complex, which triggers close apposition and subsequent fusion of synaptic vesicles with plasma membrane in living cells, was replaced by membrane-anchored complementary DNA strands. The artificial exocytosis was triggered following the addition of calcium ions, and it closely approximated the events in PC12 excretory cells. In general, the idea of the artificial SNARE complexes is based on anti-parallel hybrids of lipophilic DNA, which means that the oligonucleotide anchored by the 5' end hybridizes with its counterpart anchored by the 3' end in a zipper mode. This pulls the two membranes into close proximity and initiates lipid mixing (Fig. 1 B), although inner monolayer

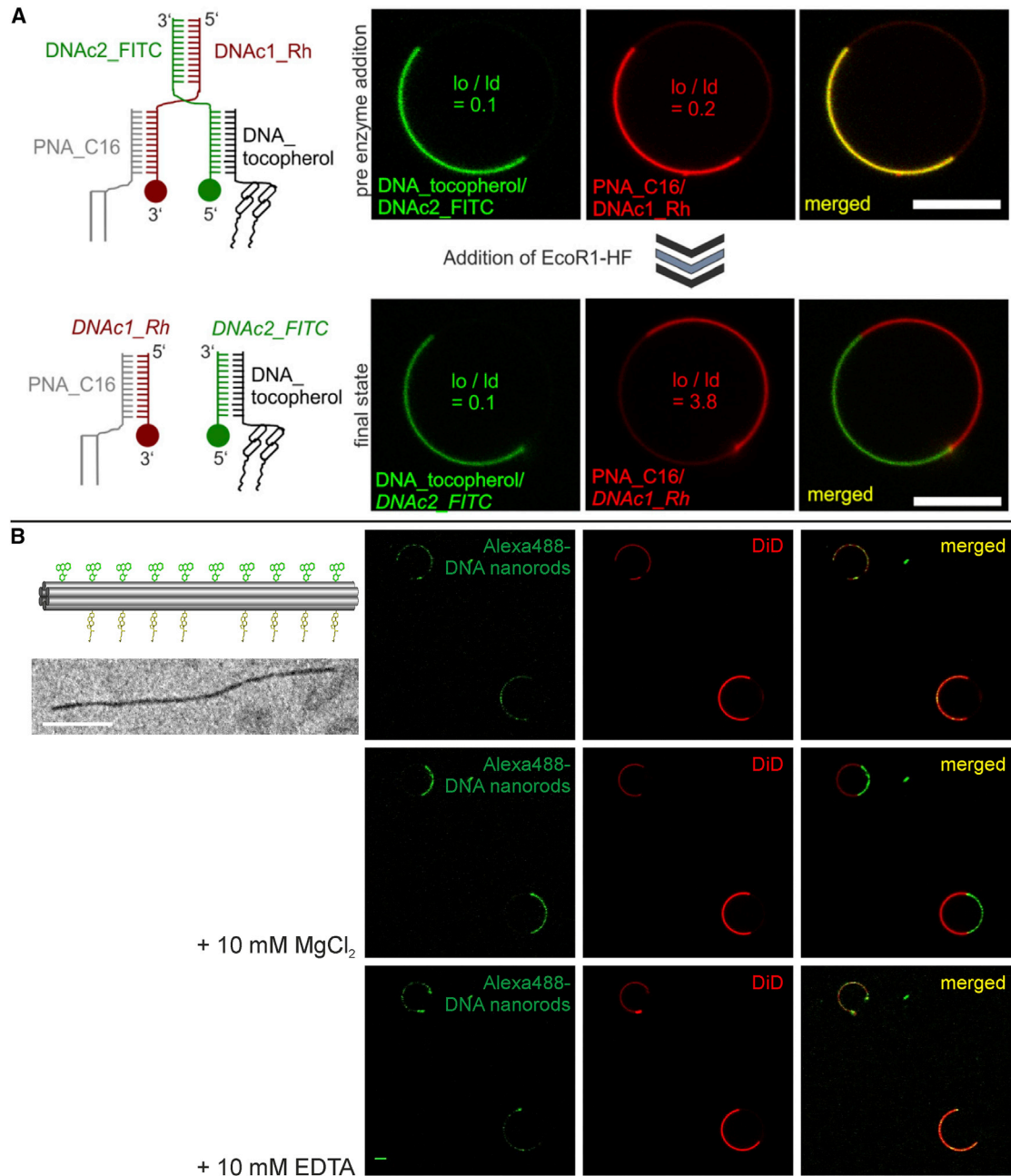


FIGURE 2 Switchable partitioning of lipophilic DNA structures on Ld/Lo membrane domains. (A) Oligo DNA/PNA complex colocalized as four-component complexes in the Ld domain, but after cleavage of the complex with restriction endonuclease (EcoR1-HF) the resulting amphiphatic components separated from each other. Ratios between the fluorescence intensities in the Lo and Ld phases are given (reproduced with permission from (40), copyright 2012 American Chemical Society). (B) Origami DNA nanorods partition into the Ld phase (marked with DiD), but after addition of Mg^{2+} ions they translocate into the Lo phase, which is fully reversible after removal of the magnesium (with EDTA). Scale bars correspond to 10 μm .

mixing was found to be usually highly restricted (23,43). On the other hand, content mixing can reach the level of ~15%, but in most cases it is even less efficient. These data could be explained by the fact that most of the zippering interactions do not go beyond formation of the membrane hemifusion state (44), and substantial leakage of content may occur during the fusion processes (23,43,45). Recent technical advances enabled time-resolved observations of lipid mixing

and content release of vesicles interacting with a surface supporting lipid bilayer at the single-vesicle level (44,46). It appeared that such processes are not only influenced by the lipid composition of the interacting membranes (with inverted-cone shaped lipids, e.g., 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine, having a key role) (47), but also by DNA properties such as linker sequences (45) and sequence features of zippers (23). The strength of DNA

anchoring to the membrane is also an important factor, as could be seen in the case of cholesterol anchors. Bivalent DNA molecules readily mediate membrane fusion processes, whereas single cholesterol moieties appeared to be much less efficient, due to their relatively high exchange between adjacent membranes (43).

However, the use of lipophilic DNA oligonucleotides for synthetic biology is limited, particularly due to structural aspects. The simplest way to create more complex molecules can be based on the membrane-assisted assembly of pseudohexagonal structures by sequential recruitment of complementary sequences (Fig. 1 C). Here, single cholesterol-modified oligonucleotides (21) or multifunctional porphyrin moieties (48) exploit unique features of DNA building blocks and amphipathic self-assembly. This soft attachment promotes free diffusion of the structures on the membrane surface and facilitates reversibility of assembly with heat-induced self-repair. A recent approach further reported small membrane-anchored 3D-cages, which upon strand displacement could unload DNA-fluorophores, assemble/disassemble into dimers/monomers, or even detach from the membrane. These features allow programmable dynamic control of binding and signaling events attributed to living cells (49).

Complex lipophilic DNA origami nanostructures

The first DNA origami nanostructures designed to interact with lipid bilayers were simple stiff 3D nanorods in the form of six-helix bundles (6HB) (50). These structures had cholesteryl-TEG anchors on one facet along the bundle and fluorescent labels positioned on the opposite site. It appeared that such membrane anchors are necessary and sufficient to attract DNA nanorods to freestanding lipid bilayers, virtually independently of the headgroup character of the phospholipids they consist of at ionic strength close to physiological. Similar structures without cholesteryl-TEG anchors appeared to bind only membranes having a positive charge (i.e., with DOTAP), particularly at low salt concentrations, which is expected for a negatively charged polyelectrolyte like DNA (51). Most recently, the weak selectivity of cholesteryl-anchored origami DNA structures with respect to membranes containing differently charged phospholipids at physiological ionic strength was shown using arrays of supported lipid bilayer patches (52). Electrostatic DNA-membrane interactions may also be affected by the phase behavior of the membrane. Double-stranded DNA was observed to preferentially bind to the L_0 domains, due to higher surface charge density of the lipids (53). In the case of cholesteryl-TEG 6HBs on GUVs composed of DOPC, sphingomyelin (or 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine), and cholesterol, strong partitioning into the L_d phase was found in the absence of magnesium or calcium ions (50). The addition of 10 mM of either of these ions changed the situation dramatically, and the nanostructures were strongly enriched in the L_0 domain. Of

importance, the partitioning could be switched back by chelation of divalent ions with EDTA (Fig. 2 B), and the membrane-anchored nanorods could be arrested in the L_d phase after enrichment of the latter with DOTAP.

The switching principle could also be realized at the level of folding DNA nanostructures via aggregation of cholesterol modifications (54). Here, the sandwich-like DNA origami with multiple cholesteryls hidden in the interior unfolded upon binding to lipid membranes. Combined with a lock and key mechanism, such structures can respond to specific molecular signals (e.g., DNA sequences), thus adding context-dependence to the membrane attachment. Another advanced example of switchable DNA nanostructures is the reversible assembly and disassembly of cholesterol-modified DNA origami-based hexagons bearing azobenzene moieties on a supported lipid bilayer (55). The hexagonal dimers disassemble upon ultraviolet irradiation, and reassemble again after visible light irradiation; this photoswitching process could be monitored by high-speed atomic force microscopy (Fig. 3 A).

Generally, it is of great interest how biological membranes organize molecules attached to their surfaces. It has been argued that the isotropic-nematic transition of strongly elongated membrane-deforming proteins in the 2D environment of lipid bilayers is a key step in membrane-mediated self-organization of these proteins, a process crucial for their biological functions (56). A simple representation of such a phase transition has been recently introduced using origami DNA nanoneedles functionalized with cholesteryl-TEG anchors (57). Selective fluorescence labeling of the nanoneedles allowed measuring their translational and rotational diffusion on freestanding lipid membranes via fluorescence correlation spectroscopy. At low DNA nanoneedle densities, the values of translational and rotational diffusion coefficients were $1.48 \mu\text{m}^2\text{s}^{-1}$ and $39 \text{rad}^2\text{s}^{-1}$, but both mobilities were strongly suppressed upon an increase in the particle density, and at surface densities approaching isotropic-nematic transition diffusion coefficients that were reduced by a factor of ~ 2.5 and ~ 100 , respectively. Thus, a clear isotropic-nematic transition on lipid membranes could be observed and characterized.

The DNA origami approach opens up new possibilities to build sophisticated biomimetic systems on membranes. It can not only be tuned by the wide range of functional modulations of membrane-active nanostructures in the exact configuration of their anchors and interfaces presented to the lipid bilayer, but also via membrane composition and buffer conditions (50,57,58). On top of that, operating with appropriate sets of oligonucleotides, one can induce reversible exchange of fluorescent moieties between membrane-anchored origami DNA and bulk molecules, initiate the assembly of the nanostructures in the plane of the bilayer, or enhance their dissociation into the solution (58). Even non-anchored DNA origami nanostructures can form regular 2D arrays on supported lipid bilayers (59). Here, weak

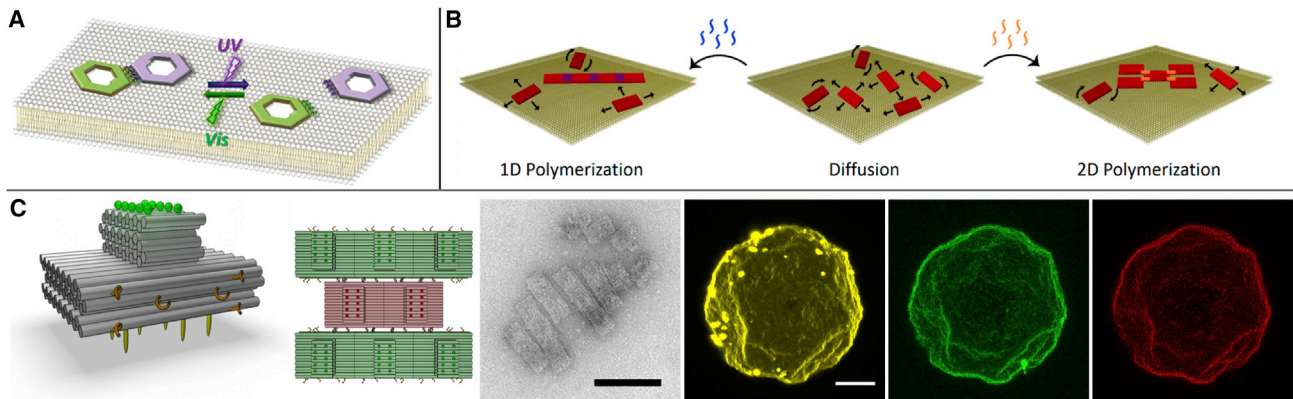


FIGURE 3 Controllable assembly of lipophilic origami DNA nanostructures on lipid membranes. (A) Origami DNA hexagons with photoresponsive modifications were reversibly assembled or disassembled upon irradiation with light of a different wavelength (reproduced with permission from (73), copyright 2015 American Chemical Society). (B) Origami DNA blocks were polymerized into one-dimensional or two-dimensional complexes via specific DNA oligonucleotides depicted in blue or orange, respectively (reproduced with permission from (60), copyright 2015 American Chemical Society). (C) Two populations of amphipathic origami DNA monoliths (labeled either with Alexa488 (green) or Alexa647 (red)) form regular array, as shown on a transmission electron microscopy (TEM) image (scale bar is 100 nm), which at high surface densities deform a GUV (scale bar is 10 μm) (reproduced with permission from (61), copyright 2015 John Wiley and Sons).

Mg^{2+} -mediated interactions between zwitterionic lipids and DNA drive electrostatic adsorption to the membrane, whereas connection between origami monomers was for instance mediated by blunt-end stacking interactions. Moreover, membrane-assisted oligomerization of DNA origami can be used to mimic the function of membrane-sculpting proteins, as recently shown (60,61). For example, freely diffusing membrane-anchored origami DNA structures were assembled into various superstructures in a programmed manner by the addition of various sets of connector oligonucleotides (60) (Fig. 3 B). The self-assembly was achieved for rectangular-shaped nanostructures as well as for molecules that mimicked the “Y” shape of clathrin triskelion. Formation of arrays of rectangular DNA origami on the surface of liposomes resulted in shape deformation or even disruption of the vesicles. Membrane-sculpting activity at a larger scale was reported for slightly differently designed DNA nanoparticles (61) (Fig. 3 C). Here, the DNA origami molecules with flat membrane-binding interface decorated with cholesterol anchors had sticky oligonucleotide overhangs on their side facets that enabled lateral interactions, as in the case of BAR domain superfamily proteins (62). Introducing a two-unit system, where each unit only differs on the fluorescent marker and sequence of sticky oligonucleotides, allowed controlling the interactions between individual molecules and their spatial arrangements. These structures colocalized and codiffused on freestanding membranes, being able to form tight and regular (brick-like) 2D scaffolds, and, at high surface densities, promoted extensive planar deformations of giant vesicles at physiological conditions. This could be directly related to deformations of GUVs reported for the flat PinkBAR domain (63).

Furthermore, even more sophisticated DNA origami-based synthetic systems to imitate key biological functions

on membranes encompass artificial lipid bilayer-embedded channels. One of such channel-like engineered nanostructures consisted of 54 parallel DNA helices, of which six located at the center penetrated through the membrane (64) (Fig. 4, A and B). This hemolysin-inspired channel indeed exhibited gating properties similar to natural ion channels. The channel was anchored to the lipid bilayer by multiple cholesterol anchors, but the penetrating part did not contain any hydrophobic modifications, enforcing a rearrangement of lipids and the formation of a hydrophilic pore around the DNA channel. Another design was based on smaller structures with hydrophobic modifications on the outer surface of the transmembrane part that exactly matched the hydrophobic thickness of the lipid bilayer. This enabled a stable channel insertion into the membrane and a tight sealing with surrounding lipid environment (65,66). Most recently, the membrane channels of 6HB architecture were shown to switch between open and closed states in a voltage-dependent manner (67). Such DNA nanopores appeared to target cellular membranes and to exert cytotoxic effects, opening up the possibility to create custom-designed biomedical devices after functionalization with targeting molecules (68). Lately, an alternative approach to the DNA origami nanopores has been described with amphipathic DNA tiles (69). Using this approach, a 45 kDa DNA tile with two cholesterol-tags for membrane-anchoring and a subnanometer-sized channel was produced. The structure proved to bind to DOPC and 1,2-diphytanoylo-*sn*-glycero-3-phosphocholine bilayers and additionally to enable ion conductance through lipid bilayers.

Finally, another very popular context for DNA origami structures in the field of lipid membranes is the development of hybrid delivery vesicles for biomedical purposes. A

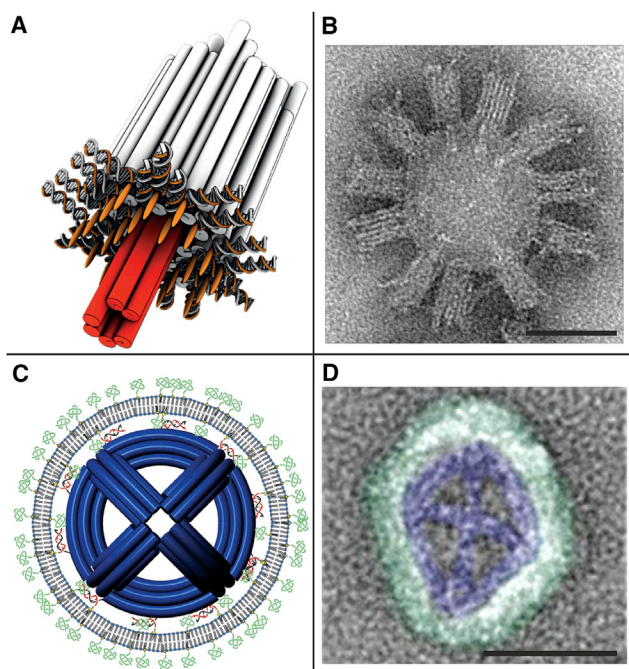


FIGURE 4 A variety of membrane-anchored origami DNA nanostructures. (A) Cholesteryl-anchored (orange) origami DNA channel consisting of a barrel-like cap (white) and a transmembrane stem (red), which spontaneously dock to liposomes, as shown on a TEM image (B) (reproduced with permission from (64), copyright 2012 The American Association for the Advancement of Science). (C) Virus-inspired membrane-enveloped DNA nanostructures for biomedical applications; color-enhanced TEM image (D) represents the DNA nanostructure (dark blue) tightly wrapped with unilamellar membrane (light blue) (reproduced with permission from (70) copyright 2014 American Chemical Society). Scale bars 50 nm.

recent investigation described a virus-inspired membrane-encapsulated origami nanostructure (70). There, a DNA octahedron, with protruding ssDNA handles on its outer side, was able to bind to lipid-conjugated oligonucleotides and be enveloped by a lipid bilayer (Fig. 4, C and D). Interestingly, the authors registered an improved *in vivo* pharmacokinetic bioavailability in mice of the membrane enveloped DNA octahedron when compared to the nonenveloped origami version. Moreover, to assess the prospect of using such DNA nanodevices for biomedical and pharmacological applications, the sensitivity of bare DNA nanostructures to the *in vitro* tissue culture environment was further assessed, to identify which cell culture media ensure DNA origami integrity (71). In combination with a more elaborate design based on switchable cages for the transport of molecular payloads (72), DNA origami-lipid vesicle delivery particles may, in the future, provide suitable options to perform drug delivery in a highly efficient, cell-specific, and targeted manner.

CONCLUSIONS

DNA nanotechnology is a rapidly evolving field promising unique advantages for controllable nanoscale engineering of biomolecules and biologically relevant processes. The

compelling self-assembly properties of DNA have opened up a wide spectrum of biological applications besides its original role as information storage. Of particular recent interest is the engineering of DNA-based tools for lipid membrane biophysics. The possibility to functionalize oligonucleotides with cholesteryl or other lipophilic moieties is of major relevance to enable the implementation of DNA nanostructures into lipidic environments. Considering the partitioning properties of membrane-anchoring tags and the specificity of DNA interactions, it is thus possible to build all kinds of dynamic and functional membrane-active modules. In addition, since the development of the DNA origami technique by Rothemund in 2006 (5), the complexity of DNA-based nanodevices has dramatically increased. Biomimetic devices performing advanced biofunctional tasks can now be conceived and created. As an illustrative example, we can particularly highlight origami structures mimicking membrane-sculpting proteins, e.g., involved in endocytosis. The capability of those artificial nanostructures to undergo isotropic-nematic transitions on lipid bilayers (57), to show membrane-assisted oligomerization (60) and most notably, to macroscopically scaffold and deform giant vesicles (61), is of great biological interest. Although these phenomena have been all demonstrated with planar origami structures, a next key step would be to develop curved DNA origami amphipathic objects that mimic the properties of coat (e.g., clathrin) or BAR domain proteins and, in this sense, investigate in a controllable manner the influence of structure, in particular, curvature on membrane bending. Another future hallmark for such nanostructures would be the implementation of externally controllable conformational switches that, in a minimalistic way, allow modeling the structural dynamics involved in biological membrane transformation and curvature recognition events.

In summary, DNA nanotechnology appears as an extremely valuable and unrivaled bioengineering tool. The biocompatibility of DNA, its sequence specificity, and controllable self-assembly makes this molecule particularly exciting for nanofabrication of biomimetic components. When decorated with lipid moieties, DNA nanodevices can be excellent artificial systems for tackling and recreating minimal tasks of membrane-embedded proteins. These intrinsic properties make DNA nanotechnology an optimal toolkit for reconstitution of biophysical processes in bottom-up synthetic biology.

AUTHOR CONTRIBUTIONS

The article was written through contributions of all authors. All authors have given approval to the final version of the article.

ACKNOWLEDGMENTS

This work has been supported in collaborative research centers of the Deutsche Forschungsgemeinschaft (DFG), begun in TRR83, and continued in

SFB863. A.C. acknowledges the support by Polish Ministry of Science and Higher Education (Iuventus Plus project IP2014 007373) H.G.F. acknowledges the receipt of a Humboldt Research Fellowship. Further support was given by the Max Planck Society to P.S.

REFERENCES

- Schwille, P. 2011. Bottom-up synthetic biology: engineering in a tinkerer's world. *Science*. 333:1252–1254.
- Czogalla, A., M. Grzybek, ..., U. Coskun. 2014. Validity and applicability of membrane model systems for studying interactions of peripheral membrane proteins with lipids. *Biochim. Biophys. Acta*. 1841:1049–1059.
- Beales, P. A., and T. K. Vanderlick. 2007. Specific binding of different vesicle populations by the hybridization of membrane-anchored DNA. *J. Phys. Chem. A*. 111:12372–12380.
- Douglas, S. M., H. Dietz, ..., W. M. Shih. 2009. Self-assembly of DNA into nanoscale three-dimensional shapes. *Nature*. 459:414–418.
- Rothemund, P. W. 2006. Folding DNA to create nanoscale shapes and patterns. *Nature*. 440:297–302.
- Castro, C. E., F. Kilchherr, ..., H. Dietz. 2011. A primer to scaffolded DNA origami. *Nat. Methods*. 8:221–229.
- Dietz, H., S. M. Douglas, and W. M. Shih. 2009. Folding DNA into twisted and curved nanoscale shapes. *Science*. 325:725–730.
- Simmel, F. C. 2012. DNA-based assembly lines and nanofactories. *Curr. Opin. Biotechnol.* 23:516–521.
- Jones, M. R., N. C. Seeman, and C. A. Mirkin. 2015. Nanomaterials. Programmable materials and the nature of the DNA bond. *Science*. 347:1260901–1–126091–11.
- Lin, C., Y. Liu, ..., H. Yan. 2006. DNA tile based self-assembly: building complex nanoarchitectures. *ChemPhysChem*. 7:1641–1647.
- Wei, B., M. Dai, and P. Yin. 2012. Complex shapes self-assembled from single-stranded DNA tiles. *Nature*. 485:623–626.
- Ke, Y., L. L. Ong, ..., P. Yin. 2012. Three-dimensional structures self-assembled from DNA bricks. *Science*. 338:1177–1183.
- Börjesson, K., J. Wiberg, ..., B. Albinsson. 2010. Functionalized nanostructures: redox-active porphyrin anchors for supramolecular DNA assemblies. *ACS Nano*. 4:5037–5046.
- Pfeiffer, I., and F. Höök. 2004. Bivalent cholesterol-based coupling of oligonucleotides to lipid membrane assemblies. *J. Am. Chem. Soc.* 126:10224–10225.
- Bunge, A., A. Kurz, ..., D. Huster. 2007. Lipophilic oligonucleotides spontaneously insert into lipid membranes, bind complementary DNA strands, and sequester into lipid-disordered domains. *Langmuir*. 23:4455–4464.
- Bunge, A., M. Loew, ..., D. Huster. 2009. Lipid membranes carrying lipophilic cholesterol-based oligonucleotides—characterization and application on layer-by-layer coated particles. *J. Phys. Chem. B*. 113:16425–16434.
- Gambinossi, F., M. Banchelli, ..., P. Baglioni. 2010. Modulation of density and orientation of amphiphilic DNA anchored to phospholipid membranes. I. Supported lipid bilayers. *J. Phys. Chem. B*. 114:7338–7347.
- Banchelli, M., F. Gambinossi, ..., P. Baglioni. 2010. Modulation of density and orientation of amphiphilic DNA on phospholipid membranes. II. Vesicles. *J. Phys. Chem. B*. 114:7348–7358.
- Banchelli, M., F. Betti, ..., P. Baglioni. 2008. Phospholipid membranes decorated by cholesterol-based oligonucleotides as soft hybrid nanostructures. *J. Phys. Chem. B*. 112:10942–10952.
- Cao, Z., R. Tong, ..., Y. Lu. 2009. Reversible cell-specific drug delivery with aptamer-functionalized liposomes. *Angew. Chem. Int. Ed. Engl.* 48:6494–6498.
- Bombelli, F. B., F. Betti, ..., D. Berti. 2009. Closed nanoconstructs assembled by step-by-step ss-DNA coupling assisted by phospholipid membranes. *Soft Matter*. 5:1639–1645.
- Beales, P. A., and T. K. Vanderlick. 2009. Partitioning of membrane-anchored DNA between coexisting lipid phases. *J. Phys. Chem. B*. 113:13678–13686.
- Chan, Y. H., B. van Lengerich, and S. G. Boxer. 2008. Lipid-anchored DNA mediates vesicle fusion as observed by lipid and content mixing. *Biointerphases*. 3:FA17–FA21.
- Loew, M., R. Springer, ..., A. Arbuzova. 2010. Lipid domain specific recruitment of lipophilic nucleic acids: a key for switchable functionalization of membranes. *J. Am. Chem. Soc.* 132:16066–16072.
- Dave, N., and J. Liu. 2012. Biomimetic sensing based on chemically induced assembly of a signaling DNA aptamer on a fluid bilayer membrane. *Chem. Commun. (Camb.)*. 48:3718–3720.
- Hadorn, M., and P. Eggenberger Hotz. 2010. DNA-mediated self-assembly of artificial vesicles. *PLoS One*. 5:e9886.
- Loew, M., J. Kang, ..., A. Arbuzova. 2009. Controlled assembly of vesicle-based nanocontainers on layer-by-layer particles via DNA hybridization. *Small*. 5:320–323.
- Jakobsen, U., A. C. Simonsen, and S. Vogel. 2008. DNA-controlled assembly of soft nanoparticles. *J. Am. Chem. Soc.* 130:10462–10463.
- Chung, M., B. J. Koo, and S. G. Boxer. 2013. Formation and analysis of topographical domains between lipid membranes tethered by DNA hybrids of different lengths. *Faraday Discuss.* 161:333–345, discussion 419–359.
- Chan, Y. H., P. Lenz, and S. G. Boxer. 2007. Kinetics of DNA-mediated docking reactions between vesicles tethered to supported lipid bilayers. *Proc. Natl. Acad. Sci. USA*. 104:18913–18918.
- Benkoski, J. J., and F. Höök. 2005. Lateral mobility of tethered vesicle-DNA assemblies. *J. Phys. Chem. B*. 109:9773–9779.
- Gunnarsson, A., P. Jönsson, ..., F. Höök. 2009. Kinetic and thermodynamic characterization of single-mismatch discrimination using single-molecule imaging. *Nucleic Acids Res.* 37:e99.
- Granéli, A., M. Edvardsson, and F. Höök. 2004. DNA-based formation of a supported, three-dimensional lipid vesicle matrix probed by QCM-D and SPR. *ChemPhysChem*. 5:729–733.
- Beales, P. A., and T. K. Vanderlick. 2009. DNA as membrane-bound ligand-receptor pairs: duplex stability is tuned by intermembrane forces. *Biophys. J.* 96:1554–1565.
- Simons, K., and W. L. Vaz. 2004. Model systems, lipid rafts, and cell membranes. *Annu. Rev. Biophys. Biomol. Struct.* 33:269–295.
- Bacia, K., D. Scherfeld, ..., P. Schwille. 2004. Fluorescence correlation spectroscopy relates rafts in model and native membranes. *Biophys. J.* 87:1034–1043.
- Weise, K., G. Triola, ..., R. Winter. 2009. Influence of the lipidation motif on the partitioning and association of N-Ras in model membrane subdomains. *J. Am. Chem. Soc.* 131:1557–1564.
- Kurz, A., A. Bunge, ..., A. Herrmann. 2006. Lipid-anchored oligonucleotides for stable double-helix formation in distinct membrane domains. *Angew. Chem. Int. Ed. Engl.* 45:4440–4444.
- Beales, P. A., J. Nam, and T. K. Vanderlick. 2011. Specific adhesion between DNA-functionalized “Janus” vesicles: size-limited clusters. *Soft Matter*. 7:1747–1755.
- Schade, M., A. Knoll, ..., A. Arbuzova. 2012. Remote control of lipophilic nucleic acids domain partitioning by DNA hybridization and enzymatic cleavage. *J. Am. Chem. Soc.* 134:20490–20497.
- Rodríguez-Pulido, A., A. I. Kondrachuk, ..., A. Herrmann. 2013. Light-triggered sequence-specific cargo release from DNA block copolymer-lipid vesicles. *Angew. Chem. Int. Ed. Engl.* 52:1008–1012.
- Simonsson, L., M. E. Kurczyk, ..., A. S. Cans. 2012. A functioning artificial secretory cell. *Sci. Rep.* 2:824.
- Stengel, G., L. Simonsson, ..., F. Höök. 2008. Determinants for membrane fusion induced by cholesterol-modified DNA zippers. *J. Phys. Chem. B*. 112:8264–8274.
- van Lengerich, B., R. J. Rawle, ..., S. G. Boxer. 2013. Individual vesicle fusion events mediated by lipid-anchored DNA. *Biophys. J.* 105:409–419.

45. Chan, Y. H., B. van Lengerich, and S. G. Boxer. 2009. Effects of linker sequences on vesicle fusion mediated by lipid-anchored DNA oligonucleotides. *Proc. Natl. Acad. Sci. USA*. 106:979–984.
46. Rawle, R. J., B. van Lengerich, ..., S. G. Boxer. 2011. Vesicle fusion observed by content transfer across a tethered lipid bilayer. *Biophys. J.* 101:L37–L39.
47. Stengel, G., R. Zahn, and F. Höök. 2007. DNA-induced programmable fusion of phospholipid vesicles. *J. Am. Chem. Soc.* 129:9584–9585.
48. Börjesson, K., E. P. Lundberg, ..., B. Albinsson. 2011. Soft-surface DNA nanotechnology: DNA constructs anchored and aligned to lipid membrane. *Angew. Chem. Int. Ed. Engl.* 50:8312–8315.
49. Conway, J. W., C. Madwar, ..., H. F. Sleiman. 2014. Dynamic behavior of DNA cages anchored on spherically supported lipid bilayers. *J. Am. Chem. Soc.* 136:12987–12997.
50. Czogalla, A., E. P. Petrov, ..., P. Schwille. 2013. Switchable domain partitioning and diffusion of DNA origami rods on membranes. *Faraday Discuss.* 161:31–43, discussion 113–150.
51. Herold, C., P. Schwille, and E. P. Petrov. 2010. DNA condensation at freestanding cationic lipid bilayers. *Phys. Rev. Lett.* 104:148102.
52. Hirtz, M., J. Brglez, ..., C. M. Niemeyer. 2015. Selective binding of DNA origami on biomimetic lipid patches. *Small*. 11:5752–5758.
53. Kato, A., A. Tsuji, ..., K. Yoshikawa. 2010. Phase separation on a phospholipid membrane inducing a characteristic localization of DNA accompanied by its structural transition. *J. Phys. Chem. Lett.* 1:3391–3395.
54. List, J., M. Weber, and F. C. Simmel. 2014. Hydrophobic actuation of a DNA origami bilayer structure. *Angew. Chem. Int. Ed. Engl.* 53:4236–4239.
55. Suzuki, Y., M. Endo, ..., H. Sugiyama. 2014. Dynamic assembly/disassembly processes of photoresponsive DNA origami nanostructures directly visualized on a lipid membrane surface. *J. Am. Chem. Soc.* 136:1714–1717.
56. Simunovic, M., C. Mim, ..., G. A. Voth. 2013. Protein-mediated transformation of lipid vesicles into tubular networks. *Biophys. J.* 105:711–719.
57. Czogalla, A., D. J. Kauert, ..., E. P. Petrov. 2015. DNA origami nano-needles on freestanding lipid membranes as a tool to observe isotropic-nematic transition in two dimensions. *Nano Lett.* 15:649–655.
58. Johnson-Buck, A., S. Jiang, ..., N. G. Walter. 2014. DNA-cholesterol barges as programmable membrane-exploring agents. *ACS Nano*. 8:5641–5649.
59. Suzuki, Y., M. Endo, and H. Sugiyama. 2015. Lipid-bilayer-assisted two-dimensional self-assembly of DNA origami nanostructures. *Nat. Commun.* 6:8052.
60. Kocabey, S., S. Kemper, ..., T. Liedl. 2015. Membrane-assisted growth of DNA origami nanostructure arrays. *ACS Nano*. 9:3530–3539.
61. Czogalla, A., D. J. Kauert, ..., P. Schwille. 2015. Amphipathic DNA origami nanoparticles to scaffold and deform lipid membrane vesicles. *Angew. Chem. Int. Ed. Engl.* 54:6501–6505.
62. Frost, A., R. Perera, ..., V. M. Unger. 2008. Structural basis of membrane invagination by F-BAR domains. *Cell*. 132:807–817.
63. Pykäläinen, A., M. Boczkowska, ..., P. Lappalainen. 2011. Pinkbar is an epithelial-specific BAR domain protein that generates planar membrane structures. *Nat. Struct. Mol. Biol.* 18:902–907.
64. Langecker, M., V. Arnaut, ..., F. C. Simmel. 2012. Synthetic lipid membrane channels formed by designed DNA nanostructures. *Science*. 338:932–936.
65. Burns, J. R., E. Stulz, and S. Howorka. 2013. Self-assembled DNA nanopores that span lipid bilayers. *Nano Lett.* 13:2351–2356.
66. Burns, J. R., K. Göpfrich, ..., S. Howorka. 2013. Lipid-bilayer-spanning DNA nanopores with a bifunctional porphyrin anchor. *Angew. Chem. Int. Ed. Engl.* 52:12069–12072.
67. Seifert, A., K. Göpfrich, ..., S. Howorka. 2015. Bilayer-spanning DNA nanopores with voltage-switching between open and closed state. *ACS Nano*. 9:1117–1126.
68. Burns, J. R., N. Al-Juffali, ..., S. Howorka. 2014. Membrane-spanning DNA nanopores with cytotoxic effect. *Angew. Chem. Int. Ed. Engl.* 53:12466–12470.
69. Göpfrich, K., T. Zettl, ..., U. F. Keyser. 2015. DNA-tile structures induce ionic currents through lipid membranes. *Nano Lett.* 15:3134–3138.
70. Perrault, S. D., and W. M. Shih. 2014. Virus-inspired membrane encapsulation of DNA nanostructures to achieve in vivo stability. *ACS Nano*. 8:5132–5140.
71. Hahn, J., S. F. Wickham, ..., S. D. Perrault. 2014. Addressing the instability of DNA nanostructures in tissue culture. *ACS Nano*. 8:8765–8775.
72. Douglas, S. M., I. Bachelet, and G. M. Church. 2012. A logic-gated nanorobot for targeted transport of molecular payloads. *Science*. 335:831–834.
73. Suzuki, Y., M. Endo, and H. Sugiyama. 2015. Mimicking membrane-related biological events by DNA origami nanotechnology. *ACS Nano*. 9:3418–3420.