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Engineering Lipid Membranes with Programmable DNA Nanostructures

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Abstract

Lipid and DNA are abundant biomolecules with critical functions in cells. The water-insoluble, amphipathic lipid molecules are best known for their roles in energy storage (e.g. as triglyceride), signaling (e.g. as sphingolipid), and compartmentalization (e.g. by forming membrane-enclosed bodies). The soluble, highly negatively charged DNA, which stores cells' genetic information, has proven to be an excellent material for constructing programmable nanostructures in vitro thanks to its self-assembling capabilities. These two seemingly distant molecules make contact within cell nuclei, often via lipidated proteins, with proposed functions of modulating chromatin structures. Carefully formulated lipid/DNA complexes are promising reagents for gene therapy. The past few years saw an emerging research field of interfacing DNA nanostructures with lipid membranes, with an overarching goal of generating DNA/lipid hybrid materials that possess novel and controllable structure, dynamics, and function. An arsenal of DNA-based tools has been created to coat, mold, deform, and penetrate lipid bilayers, affording us the ability to manipulate membranes with nanoscopic precision. These membrane engineering methods not only enable quantitative biophysical studies, but also open new opportunities in synthetic biology (e.g. artificial cells) and therapeutics (e.g. drug delivery).

Graphical Abstract

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Conflict of Interest:

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In cells, sophisticated protein machineries modulate membrane structure and dynamics. However, evolution presents these beautiful constructs without a user manual, making them difficult to dissect and re-engineer. With cutting-edge DNA nanotechnology, scientists can now build artificial nanostructures that look and act like membrane-manipulating proteins, but with programmable functions. These tools will come in handy for basic research and biotechnology.

Keywords

DNA nanotechnology; lipid bilayers; membrane curvature; membrane remodeling; vesicles; nanopores

Introduction

Lipids are a class of amphipathic molecules with a polar or charged headgroup and a hydrophobic tailgroup. Dissolving poorly in aqueous solutions, they spontaneously cluster to shield their tails within a core while exposing their hydrophilic heads to the solvent.^[1] Figure 1a illustrates this principle using a set of lipid structures self-assembled from glycerophospholipids as examples. The lipid self-assembly outcome depends on a number of factors, including the chemical structures of the lipid constituents, the presence of substrates or auxiliary molecules (e.g. detergents and scaffolding proteins), and other self-assembly conditions such as temperature and lipid concentration. One of the most prominent lipid structures is the lipid bilayer, which features two layers (also called leaflets) of closely packed lipid molecules with their hydrophobic tails pointing towards the center of the bilayer to form the core. Lipid bilayers can be flat, delimited by protein scaffolds (Figure 1a, top center) or supported by a substrate (Figure 1a, bottom right). However, more often, they curve, either spontaneously or under external influence, to form closed structures termed liposomes or vesicles (Figure 1a, top right and bottom left). Although lipid-bilayer membranes made in different ways or with different biological origins are diverse in geometrical and physiochemical characteristics, they share a few common properties.^[2] First, because of the lipid bilayers' hydrophobic core, they are impermeable to most charged and polar molecules, including water-soluble sugars and ions. Passage of these molecules is only granted when transmembrane channels (usually lined by proteins) are present. Second, beyond a temperature characteristic to each lipid species (termed melting temperature), the

bilayer becomes fluid with rapid lateral movement of lipids within each leaflet. The exchange rate of lipids between two leaflets, with the exception of cholesterol, is negligible without the help of specialized enzymes. Third, in a membrane made of multiple lipid species, it is possible to have phase separation, where lipids segregate into domains of different thickness, stiffness, and fluidity. Other embedded molecules, such as membrane proteins, may also partition to different domains. Fourth, membranes with a given lipid composition will have a spontaneous curvature. The energy necessary to force a membrane to deviate from this intrinsic curvature is described as its bending modulus, which is primarily governed by the size, shape, and packing density of lipids in both leaflets. Fifth, other molecules in solution, such as ions, detergents, and proteins, can bind to lipid bilayers through electrostatic and hydrophobic interactions with lipid head and tail groups, respectively. These basic properties underlie the structure and function of cell membranes, many aspects of which can be faithfully recapitulated outside of cells using “model” membranes.

Like lipids, DNA is a natural, self-assembling molecule. The iconic B-form DNA comprises two DNA strands with complementary sequence that spontaneously associate through base pairing. Double-stranded DNA containing millions of base pairs folds into extremely condensed chromatin structures with the help of proteins, such as histones. Branched DNA structures also exist in cells during DNA replication and repair. In the past three decades, the field of structural DNA nanotechnology has developed techniques to build designer nanoscale architectures from DNA.^[3] The principle of constructing DNA nanostructures is simple: one only has to render a target structure with DNA double helices, connect neighboring helices with basic branched DNA motifs (e.g. immobile Holliday junctions), and program the sequence complementarity into synthetic DNA strands, which will self-assemble into the prescribed structure in aqueous solution. Because of DNA's negatively charged phosphate backbones, DNA nanostructures are generally folded and stored in solutions containing counter ions, such as sodium, magnesium, and polyamines. Once correctly folded, the DNA nanostructures maintain their structural integrity under a considerably wide range of temperature and salt concentrations. Thanks to well-established DNA thermodynamics, stringent base-pairing rules, and a number of ways to join DNA double helices, DNA nanostructures of various geometries and stiffness can be precisely engineered.^[4] Figure 1b shows a small subset of nanoscale architectures that can be folded from DNA. Using cohesive interfaces with designed shape and/or sequence complementarity, individual structural units (each with dimensions circa 10–100 nm) can be put together in a programmable way to form larger discrete structures (with dimensions of a few hundred nanometers)^[5–6] or infinite arrays (reaching hundreds of micrometers)^[7–8]. In addition to customizable shapes, DNA nanostructures are often made with addressable surfaces, meaning that each surface nucleotide, with a sequence-defined location, can be chemically addressed. This allows one to extend nucleic acid probes at specific locations to bind guest molecules (e.g. via aptamer-target binding, or hybridization with DNA strands chemically conjugated to guest molecules), effectively turning the DNA nanostructure into a template that organizes other molecules with defined stoichiometry and spatial positioning^[9]. Furthermore, DNA nanostructures that harbor structure-switching components can change shapes (e.g. expand or contract) in response to environmental cues including light,

temperature, ion concentration, and electric fields.^[10–11] This makes it possible to create synthetic DNA nanomachines with designed movement (e.g. translational and rotary motions), but also enables dynamic control of guest molecules placed on reconfigurable DNA templates.

The membranes in living cells are complex in structure and function, changing shape (a process termed membrane remodeling) throughout the cell's life cycle for environmental sensing, communication, growth, and division. Modern cell biology has revealed that the membrane structure is not merely a product of protein-mediated biochemical reactions, but also an important modulator of cell signaling and decision-making.^[12–13] Therefore, elucidating how cells generate, maintain, and change membrane structures has long been a focal point of cellular and molecular biology. Meanwhile, a major goal of biotechnology is to exploit cell membrane trafficking in order to effectively deliver reagents to cells and reprogram cell behavior.^[14] For both basic research and biomedical applications, it is desirable to have a set of versatile tools that can manufacture and manipulate membranes in precise and programmable ways. The ability of DNA nanotechnology to create nanoscale objects with well-defined attributes (size, shape, connectivity, surface chemistry, and reconfiguration pathways) provides a potential solution toward this engineering goal. In theory, one could closely associate DNA nanostructures with lipid bilayers in a desired topology, such that the programmable features can be faithfully passed from the DNA nanostructure onto its associating membrane. The interactions between membrane and DNA molecules (modified or unmodified) are well established (see reviews^[15–17]), which we summarize in Figure 1c. First, negatively charged DNA can bind to cationic and zwitterionic lipid headgroups through electrostatic interactions, which can be modulated by the surface charge density of a lipid bilayer and cation (e.g. K^+ , Mg^{2+}) concentration in solution.^[18] Theoretical modeling suggests that divalent ions promote lipid-DNA binding by bridging and reorienting the lipids' zwitterionic headgroups.^[19] Second, the DNA can be modified, on either terminus or the phosphate backbone, with hydrophobic moieties such as cholesterol, phospholipids, or ethyl-thiol groups, which spontaneously insert into the membrane. Third, with a specific ligand (e.g. aptamer, antibody) attached, DNA structures can bind to proteins embedded in lipid bilayers, thus associating with the membrane.

In the following sections, we will survey different subfields of research, highlight breakthroughs in DNA-nanotechnology-enabled membrane engineering^[20], and discuss challenges ahead for the field to reach its full potential in basic research and biotechnology.

Membrane-association behaviors of DNA nanostructures

Since the inception of structural DNA nanotechnology^[21], and especially with recent development in design principles and tools (e.g. DNA origami and caDNAno)^[22–23], a plethora of designer architectures comprised of hundreds of DNA strands (molecular weight over 5×10^3 kD) have been produced, sporting thousands of square nanometers of negatively-charged surfaces that can be selectively modified with hydrophobic moieties. However, before one can build any membrane-engineering tool with these intricate structures, it is essential to establish mechanisms to associate DNA with lipid bilayers and understand the behaviors of membrane-bound DNA assemblies. Although the binding of nucleic acids to

lipids has been studied and used for decades (e.g. lipotransfection [14, 24]), it is important to revisit this topic with a growing family of massive and complex DNA nanostructures. Experimentally, the interactions between DNA nanostructures and model membranes (e.g. supported bilayers, giant unilamellar vesicles or GUVs) can be visualized by fluorescence microscopy (including total internal reflection and confocal microscopy), transmission electron microscopy (TEM), and atomic force microscopy (AFM).

To a large extent, the conventional wisdom of DNA-lipid binding based on electrostatic and hydrophobic interactions, as briefly summarized in Figure 1c, holds up well in the realm of structural DNA nanotechnology. However, DNA nanotechnology affords programmability in the geometry, rigidity, dynamics, and chemical modification of the nanostructures, thus providing additional means to tune DNA-membrane interactions.

Electrostatic interactions between DNA and lipids are traditionally controlled by tuning lipid and buffer compositions. Since the 1980s, liposomes containing cationic lipids (e.g. DOTMA) have been used to form complexes with plasmid DNA to facilitate efficient delivery of genetic materials into cultured cells.[25–27] Conversely, membranes that contain negatively charged lipids (e.g. DOPS) and surface-crowding reagents (e.g. PEGylated lipid) weaken the affinity of DNA to membrane. It has also been shown that such affinity is enhanced by divalent cations (Ca^{2+} , Mg^{2+}) and reduced by monovalent cations (K^+ , Na^+), possibly because divalent ions can transit from the phosphate backbone of DNA to the negatively charged pole of lipid heads, while monovalent ions cannot, yet still compete for binding.[18–19] When assembled into nanostructures, the well-defined arrangement of DNA helices dictates the surface charge density. For example, a branched three-point-star DNA motif has lower charge density than a DNA-origami block composed of a dozen closely-packed double-helices, resulting in the former being a weaker binder to DOPC bilayers in the presence of the Mg^{2+} . [28–29] However, the unmodified three-point-star tile does bind to the phase-separated DPPC domains, presumably due to the tighter packing (i.e. higher charge density) of the zwitterionic phosphatidylcholine headgroups.[28]

To strengthen the association between DNA structures and lipid membrane, hydrophobic molecules can be introduced as anchors for more robust and stable binding. Lipids (including cholesterol), alpha-tocopherol, polypropylene oxide, and porphyrin have been conjugated with DNA strands to facilitate membrane attachment. Among them, the cholesterol moiety is by far the most commonly used membrane anchor, owing to its availability as a 5' - or 3' - modification (e.g. through a triethyleneglycol (TEG) spacer) during DNA synthesis. Both experimental evidence and molecular dynamic (MD) simulations suggest that the binding to zwitterionic (net neutral) membrane is more or less irreversible with a single cholesterol modified DNA structure.[30] However, negatively charged lipid headgroups can repel DNA and weaken the DNA-membrane association. Therefore, besides using counterions to mitigate this like-charge repulsion, one could increase the number of cholesterol on a DNA nanostructure to achieve stronger membrane affinity.

Schwille et al. systematically investigated the membrane binding of DNA origami structures as a function of membrane anchor number and location.[31–32] In these setups, TEG-

cholesterol can be selectively attached at any position within a 3 by 5 array spanning the entire bottom surface of a rectangular DNA-origami structure, with or without a DNA spacer between the main body of the origami and the hydrophobic moiety. Fluorescence microscopy studies on GUVs incubated with such DNA structures showed that the number and accessibility of the cholesterol strongly influence the DNA structures' membrane affinity. Specifically, when cholesterol modifications were placed proximal to the DNA rectangle (i.e. no spacer used), a minimum of two cholesterol near the corners was needed to mediate efficient GUV binding, suggesting that the membrane interactions of the hydrophobic moieties at the center of the stiff DNA rectangle were hindered. In contrast, adding ssDNA/dsDNA spacers between the cholesterol and the bottom surface of the DNA rectangle alleviated this constraint, allowing DNA rectangles with center cholesterol modifications to bind as efficiently as their corner-modified counterparts. Other studies reported similar trends^[33–34], confirming that a larger number of accessible membrane anchors on a DNA structure enhances its membrane binding. Moreover, the orientation of the DNA structures on membrane can be controlled by membrane anchor placement. For example, with linear dichroism spectra, Nordeén et al. found a hexagonal DNA nanostructure (10 bp/edge) labeled with one glyceryl-bis-C16-hexaethyleneglycol stood perpendicular to the membrane, whereas dual-lipid-labeling at opposite corners helped the structure lay flat on the lipid surface.^[35–36]

Once anchored to the membrane, DNA structures remain bound until their membrane anchors (e.g. cholesterol) are removed or the bilayer is disrupted by detergent. When lipid bilayers are fluid, membrane-bound DNA structures exhibit dynamic behaviors much like other macromolecules on lipid bilayers. Such diffusion dynamics can be quantitatively studied by fluorescence correlation/cross-correlation spectroscopy (FCS/FCCS), single-particle tracking, fluorescence recovery after photobleaching (FRAP) and high-speed AFM (HS-AFM).

As shown in Table 1, diffusion coefficients of the membrane-anchored DNA-origami structures on lipid membrane were in the range of 0.1–2 $\mu\text{m}^2/\text{s}$, similar to those of the lipid-linked peripheral proteins on supported bilayers (0.05–2.6 $\mu\text{m}^2/\text{s}$).^[33, 37] The diffusion constant depends on the fluidity of the membrane, number of membrane anchors, surface coverage rate, and size of the DNA nanostructures. In addition to the translational diffusion, rotational motion of high aspect ratio DNA structures on membrane have also been quantified. By comparing the measured diffusion rate of a 0.42- μm -long DNA rod with a fluorescent label at its end versus at the center, the rod's rotational diffusion coefficient was calculated to be $68 \pm 3 \text{ rad}^2 \text{ s}^{-1}$. Moreover, DNA nanostructures have been shown to reversibly partition between phase-separated domains (e.g., liquid-disordered (L_d), liquid-ordered (L_o)) of multicomponent membranes (Figure 2a). The partition selectivity of a DNA structure is modulated by the chemical identity of its membrane anchors (e.g. cholesterol, tocopherol), the membrane's lipid composition, the charge density of the DNA construct, and the solution's ionic strength, which collectively determine the electrostatic and hydrophobic interactions between the DNA and membrane.^[15–16] For example, on a phase-separated GUV membrane made of DOPC, sphingomyelin and cholesterol, cholesterol-modified DNA-origami rods mostly reside in L_d phase in the absence of Mg^{2+} , likely due to cholesterol's tendency to fill the packing defects between unsaturated lipid tails, whereas the

addition of 10 mM Mg^{2+} leads to translocation of the rods into L_o phase, where the densely packed zwitterionic lipid heads can attract DNA electrostatically with the help of divalent cations.^[38]

DNA nanostructures modified with amphipathic anchors can have strong tendencies to aggregate by way of hydrophobic interactions. The severity of the aggregation varies, depending on the type and density of anchor, DNA structure concentration, and the presence of other amphipathic molecules. These aggregation dynamics were used by Simmel et al. to construct a dynamic DNA origami “clamshell” (Figure 2c).^[39] There, up to 28 cholesterol molecules were attached to one side of an origami sheet to mediate spontaneous closure of the DNA clamshell, which could open in the presence of surfactant (Tween 80) or lipid membrane. Keyser et al. reported a neat trick to mitigate aggregation of cholesterol-modified DNA nanostructures without affecting their bilayer-binding ability, namely to shield cholesterol with juxtaposed ssDNA overhangs.^[40]

It has been well established that DNA nanostructures bearing complementary oligomerization interfaces can form higher-order assemblies such as two-dimensional (2D) crystals^[8, 41–42] and quasi-crystals^[43–45] in solution and on surfaces. Because of their tunable fluidity and DNA-binding affinity, lipid bilayers provided an appealing substrate for surface-assisted DNA assembly. In 2014, Sugiyama et al. designed DNA origami hexagons that could dimerize through azobenzene-modified oligonucleotides and undergo light-induced dissociation and association (Figure 2d).^[46] The dimeric DNA hexagons (modified with cholesterol at their base) bound preferably to the L_o phase of a supported bilayer (containing sphingomyelin) on mica, where their photo-switchable assembly/disassembly was recorded by HS-AFM. In 2015, the same group used HS-AFM to study the assembly dynamics of a 2D DNA lattice on supported DOPC bilayers (Figure 2e).^[47] This time, unlabeled cross-shaped DNA origami structures were designed to assemble through blunt-end association. The dynamic growth of the 2D lattice, including lattice fusion and defect patching, were recorded with 5–10 s temporal resolution. Blocking the blunt ends of the origami monomers by ssDNA extensions led to a change of the assembly outcome from a lattice with square-shaped voids to an array of densely packed origami monomers. Similar phenomena have been reported by Sleiman et al. using smaller multi-arm DNA tiles (with or without cholesterol anchors) capable of forming well-organized arrays with various patterns on supported DOPC and DPPC bilayers (Figure 2f).^[28] In 2018, Suzuki et al. recorded the interesting phenomena of Na^+ -induced DNA-origami desorbing from L_d domains and lattice formation on L_o domain of a phase-separated bilayer.^[48] Further, Yanagisawa, Takinoue, et al. assembled an $\sim 1\text{-}\mu\text{m}$ -thick network of 3-way DNA junctions on a positively-charged membrane inside lipids-in-oil droplets, thereby stabilizing the droplets and subsequently formed GUVs similar to cytoskeleton supporting cells.^[49]

Liedl et al. designed a rigid rectangular DNA-origami block that could polymerize either in 1D or 2D on DOPC membrane, depending on the choice of connector DNA strands that crosslink the neighboring DNA blocks.^[29] They also built clathrin-inspired DNA triskelia that formed hexagonal 2D lattices on membrane. In this work, the DNA structures were attached to membranes by hybridizing their single-stranded extensions with cholesterol-modified oligonucleotides pre-anchored to the bilayers. Intriguingly, the rectangular blocks

seemed to deform lipid vesicles to some extent upon polymerization. We will review such membrane-remodeling DNA assemblages in a later section.

Scaffolding lipid bilayer self-assembly with DNA nanostructures

A long-standing challenge in membrane engineering is the generation of monodispersed liposomes of programmable sizes, shapes, and spatial arrangements. This is important for both biophysics studies (e.g. curvature-dependent protein-membrane interactions^[12, 50–52]) and biomedical applications (e.g. control the payload of drug carriers^[53–54]). Previous methods can produce homogenous liposomes of certain sizes, but lack the versatility to do so across a range of sizes or with various lipid compositions, and have limited control over vesicle geometry at the nanometer scale. On the other hand, DNA nanotechnology excels at creating customizable shapes at the same length scale of physiologically-relevant membrane structures. This provides an opportunity to generate geometrically-defined liposomes using DNA nanostructures as a template. The basic idea is to build a DNA “mold” modified with hydrophobic molecules to nucleate and scaffold lipid self-assembly, eventually coating the exterior (Figure 3a) or interior (Figure 3b) of the DNA mold surface with lipid bilayers, resulting in a liposome with its shape defined by the DNA mold.

Generally speaking, this is experimentally achieved as follows (Figure 3b): (i) design and assemble DNA-origami templates with desired geometry and dimensions; staple extensions (termed “handles”) are reserved for subsequent lipid/protein labeling; (ii) chemically conjugate hydrophobic molecules, such as a glycerophospholipid, to an “anti-handle” DNA strand with a complementary sequence to the handle strand; (iii) hybridize the lipid-modified anti-handles to the DNA templates bearing the desirable handle configuration in the presence of detergent; (iv) supply the lipid-modified DNA template with excessive lipids; and (v) remove detergent through dialysis and purify the geometry-controlled liposomes in a density gradient.

The first DNA-nanostructure-scaffolded liposome formation was demonstrated by Perrault and Shih in 2014 using the approach described above.^[55] On the outer surface of a DNA nano-octahedron (diameter ≈ 50 nm), up to 48 handles were displayed to immobilize phospholipid-modified anti-handles (Figure 3a). Here, the DNA octahedron served as an endoskeleton to template the formation of the surrounding lipid bilayer ($\sim 95\%$ DOPC, 5% of PEG-2k-PE) shell. After purification, PEGylated vesicles with a mean size of 76 nm were obtained. The membrane integrity of the vesicles was studied by treating the encapsulated DNA structures with an intercalating dye and DNase I. They found that with 48 handles, about 70–80% of the DNA structures were completely shielded from digestion by their enclosing membrane, whilst less handles per structure led to reduced protection. Most importantly, compared to the uncoated counterparts, the membrane-enclosed DNA structures showed ~ 100 -fold reduction in immunogenicity when incubated with cultured immune cells and enjoyed a ~ 10 -fold increase in circulation time when injected into mice via the tail vein. This suggests that building enveloped-virus-like particles with designable DNA/lipid formulas is a promising way to develop nanodevices that can work long hours *in vivo*.

To better define the liposome geometry, our lab built liposomes with DNA nanostructures as exoskeletal templates.^[56] We first designed four DNA-origami rings with sub-100 nm inner diameters (29, 46, 60, and 94 nm to be precise) to control the liposome size, and grafted 12–49 lipid molecules (“seeds”) onto their inner surfaces to nucleate the lipid self-assembly during detergent removal. By means of isopycnic centrifugation, we were able to enrich Saturn-like complexes with liposomes filling the DNA rings (hence highly homogenous in size), as visualized by negative-stained TEM and Cryo-EM (Figure 3b). Optionally, the DNA rings could be removed by nuclease digestion to fully expose the surface of the liposomes. Lipid grafting density was again found important to produce size-defined vesicles, as a lower number of pre-attached lipids per ring resulted in more rings harboring multiple smaller liposomes. Unlike the DNA octahedron, placing the lipid seeds on the outside of the ring led to the formation of discrete liposomes of undefined sizes or aggregates, presumably due to a lower nucleation (i.e. lipid grafting) density in 3D. By directly capturing the intermediates via TEM, we revealed three discernable stages during the DNA-templated lipid self-assembly, namely (i) the recruitment of lipid/detergent micelles by the lipid seeds, (ii) the expansion and merger of lipid bilayers following detergent removal, and (iii) the completion of liposome growth (often confined by the DNA template). In a separate study, we found that the lipid seeds can be replaced with SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins to generate proteoliposomes with defined protein stoichiometry and orientation^[57] (more discussion about this work can be found in the next section).

Mimicking biologically relevant membrane curvatures necessitates liposomes with aspherical shapes. To this end, we designed a set of DNA cages (two rings connected by four rigid pillars) with programmable dimensions and polymerization patterns (Figure 3c).^[58] These DNA cages have enabled the construction of an assortment of DNA-template shapes that in turn scaffolded the formation of liposomes with defined geometry and spatial arrangement, including a string of liposomes with controllable inter-membrane distances, width-defined lipid tubules, toroidal or helical vesicle bodies, and stacks of laterally associated “flat” membrane tubules. In addition to the versatility to generate various membrane curvatures, this method affords considerable flexibility when it comes to the membrane’s chemical composition. With a few exceptions, lipids with mixed headgroups and fatty-acid chains can be molded into the shapes prescribed by the DNA templates. Therefore, we could in theory recapitulate subcellular membrane structures in a test tube by taking advantage of this method. For example, we have recently built heterodimeric liposomes with several controlled distances between the apposed bilayers to mimic the endoplasmic reticulum/plasma membrane contact sites. These constructs facilitated the systematic analyses of distance-dependent lipid transfer mediated by proteins such as extended synaptotagmins ^[59].

As a popular alternative to liposomes, small patches of flat lipid bilayer encircled by proteins (termed nanodiscs) are commonly used as model membranes. Conventional nanodiscs are delimited by a pair of membrane scaffolding protein (MSP) derived from Apolipoprotein A1. Using length-engineered MSPs that are pre-circularized, monodispersed nanodiscs up to 50 nm can be created.^[60] To produce stable nanodiscs with larger sizes, Wagner, Shih, and colleagues used DNA-origami barrels to direct the formation of

nanodiscs^[61], which abolished the needs for MSP pre-circularization. Specifically, the interior surface of a DNA-origami barrel was first decorated with a number of nanodiscs (diameter ≈ 11 nm) scaffolded by non-circularized, DNA-conjugated MSPs. Addition of detergent and lipids with subsequent dialysis allowed for the reconstitution of a single nanodisc (diameters: ~ 45 or ~ 70 nm) within the DNA barrel (outer diameters: 60 or 90 nm), possibly by fusing pre-attached small nanodiscs. These large, DNA-corralled nanodiscs were used to crowd in membrane proteins, such as ion channels and photosynthetic reaction centers or capture a single poliovirus via membrane-displayed receptors (Figure 3d), suggesting the platform's utility in studying membrane protein structure and dynamics. This method is not unlike the aforementioned DNA-ring scaffolded liposome formation technique, in that both employed a seeding-reconstitution procedure within a confined space to obtain membrane structures with discrete, monodispersed diameters prescribed by a DNA template. Furthermore, both methods worked with a variety of lipid compositions. The use of MSP as amphipathic seeding molecules in this work, however, led to the formation of planar membranes thanks to the MSPs' ability to non-covalently join in tandem and stabilize the hydrophobic rim of a lipid bilayer. In a concurrent study, Schmidt et al. reconstituted protein-free nanodiscs (~ 15 and 30 nm in diameter) scaffolded by alkyl-chain-lined dsDNA circles (Figure 3e), which provides a cost-effective way to create size-defined planar bilayers from a handful of DNA strands.^[62]

DNA-guided membrane deformation

In the previous section, we reviewed DNA-nanotechnology-enabled liposome manufacturing methods, which allowed creation of arbitrary membrane curvatures. However, the membrane engineering toolset is not complete without membrane manipulation tools capable of altering membrane properties dynamically. In cells, membrane shapes change dramatically during physiological and pathological events, such as cytokinesis and viral infection. Such processes, collectively called membrane remodeling, are mediated by proteins that bind and deform membranes. Many membrane-remodeling proteins have been identified and structurally solved, which provides design inspiration for synthetic membrane manipulation tools.^[63–66] However, reconstituting and reengineering membrane remodeling events using recombinant proteins or cell extracts can be tricky, often due to challenges like protein folding, membrane-protein complex stability, and unknown molecular players involved in the process. DNA nanotechnology offers means to program molecular motions with spatial and temporal control, thus presenting an opportunity to build synthetic membrane-remodeling devices. In this section, we cover progress in DNA-mediated membrane fusion (a process where two separate bilayers merge into a continuous one) and shape changes. In the next section, we will focus on membrane-penetrating DNA nanopores.

Inspired by SNARE-family proteins^[67–68], which mediate nearly all fusion events in eukaryotes, researchers have built DNA-oligonucleotide-functionalized lipid vesicles, where the complementary DNA strands act as molecular glues to induce vesicle docking and/or fusion *in vitro*. The fusogenicity of such vesicles generally depends on the length, polarity, and density of DNA strands, along with the lipid composition of the membrane. For example, the zippering of two DNA species on the surfaces of separate vesicles, as demonstrated by Höök et al., led to vesicle fusion, evident from fluorescence resonance

energy transfer (FRET) studies that showed lipid mixing in both leaflets.^[69] Fusion efficiency and kinetics were found dependent on lipid composition and surface density of the DNA zippers. Another illustrative example was provided by Boxer et al., who systematically varied the designs of membrane-anchored DNA strands.^[70] They showed that when complementary DNA strands were anchored to vesicles via the same (5'- or 3'-) ends, they could mediate docking, but not fusion, confirming that the zipper-like pairing between DNA strands is necessary to bring vesicles into close proximity for fusion to occur. They also showed that adding poly-dT sequences to the membrane-proximal ends of the DNA strands enhanced vesicle docking (observed by fluorescence microscopy) while reducing the likelihood of fusion (monitored by FRET signals of lipid and content mixing).

Beyond these classic examples, modern DNA nanotechnology provides means to precisely control factors that modulate membrane fusion. Shih, Rothman, and colleagues equipped DNA-origami rings with designated copies of VAMP2 (a type of v-SNARE protein) in search of the minimal number of SNARE complexes (four- α -helix bundles formed by v-SNARE and the cognate t-SNAREs) required for fusion^[57]. The liposomes formed within these VAMP2-decorated DNA rings were subjected to a previously established single-molecule fusion assay^[71] that tracked the docking and fusion events of the vesicles on a supported lipid bilayer displaying SNAP25 and syntaxin (both t-SNAREs, Figure 4a). This work reported that 1–2 SNARE complexes were sufficient to drive lipid mixing shortly (100–150 ms) after the vesicles docked on the supported bilayer. Of note, freely diffusing, poly-dT-buffered ssDNA tethers, akin to those reported by Boxer et al., were used in this work to promote vesicle docking.

The modular and reconfigurable DNA cages that we developed to scaffold shape-defined liposomes could be used to drive controllable membrane remodeling^[58]. For example, a string of vesicles (diameters $\approx$ 30 nm), assembled inside DNA rings and initially separated by rigid DNA pillars, can be brought closer and eventually fused by dynamically shortening the pillars. Practically, we used toehold-mediated strand displacement to transform the rigid pillars into flexible ssDNA's, which acted as entropic springs to drive fusion. Partial disassembly of all pillars resulted in reduced liposome fusion efficiency. Using a similar setup, we bent DNA tubes by shortening its pillars on one side to turn straight membrane tubules inside into curved ones (Figure 4b). Thus, we showed that programmable DNA conformational changes can be used to control membrane dynamics with predictable energy input and deformation outcomes.

Many proteins deform membrane by binding and oligomerizing on the lipid bilayer, thereby stressing the membrane by insertion of amphipathic α -helices, surface crowding, and scaffolding (or protein-membrane shape coupling). Such proteins include clathrin, BAR domains, dynamin, and ESCRT-III subunits. It has been shown that DNA nanostructures outfitted with membrane anchors can be potent membrane-deforming reagents, possibly by similar mechanisms. The changes in membrane morphology are usually detectable by electron and fluorescence microscopy. Some of these deformations came as a surprise. For example, when building membrane-penetrating DNA channels (reviewed in the next section), Keyser et al. observed that at high DNA concentrations (100 nM), an 11-nm long, 6-nm wide DNA nanotube labeled with two cholesterol were capable of generating tube-

like structures from GUVs.^[72] Interestingly, even at low concentration (5 nM), a 9-nm long, 6-nm wide DNA tube with three cholesterol labels caused polymer-supported membranes to form lipid nanotubes parallel to the bilayer surface, as shown by a team led by Howorka and Piehler.^[73] On the other hand, DNA-origami structures with purposely-engineered flat or curved membrane-interacting surfaces can force their shape upon associated membranes. Liedl and Schwille independently reported that linker-strand-induced lateral assembly of flat DNA-origami blocks on the outer surfaces of SUVs and GUVs led to planar deformation (i.e. flattening) of the vesicles.^[29, 74] Subsequently, in two separate studies, Schwille and we designed curved DNA-origami structures labeled with cholesterol or amphipathic peptide for membrane tubulation.^[75–76] At high vesicle-surface coverage, these structures induced the formation of membrane tubules coated with DNA curls (Figure 4c). We showed that the end-to-end polymerization of DNA curls into nanosprings, though not required for membrane deformation, could enhance the tubulation efficiency. Although the exact mechanism of the DNA-curl-induced tubulation has yet to be fully elucidated, it is clear that the tubulation morphology and efficiency can be modulated by design parameters, such as rigidity, curvature, and membrane-anchor density of the DNA structure, as well as experimental conditions that include DNA coverage rate and tension of the membrane.^[34, 75–76] Recently, the lab of Turberfield reported the formation of networks of curved and flat cholesterol-labeled DNA-origami triskelia on supported lipid monolayers and GUVs.^[77] TEM studies showed partially-ordered, clathrin-like^[78] DNA arrays on monolayers and suggested DNA-network-induced monolayer budding. Collectively, these results suggest a promising approach to recognize and reshape pre-existing membranes on demand, potentially useful for sensing and manipulating features on cell membranes.

Transmembrane DNA nanopores

In addition to serving as a barrier that walls off the cytoplasm from the extracellular space, cell membranes need to allow molecular exchange with the environment. Typically, the transport of small molecules, such as ions, through plasma membrane is facilitated by protein nanopores, which form cylindrical structures that span across both leaflets of a lipid bilayer. Engineering artificial pores with customizable geometry, chemical modification, and an on/off switch will allow for the control of transport selectivity, which is useful for biosensing, sequencing, and building cell-like compartments. In recent years, a variety of chemically-modified DNA nanopores have been built to penetrate vesicle membranes, suspended bilayers, and cell membranes. For the broader topic of nanopore engineering, we point readers to a masterly review by Howorka.^[79] Below, we review the representative DNA nanopore designs and attempt to summarize their common engineering principles and unique features.

In 2012, Simmel, Dietz et al. reported the first membrane-penetrating DNA nanopore featuring a syringe-like design.^[80] It was made of a narrow tube (inner diameter $\approx$ 2 nm, outer diameter $\approx$ 6 nm) protruding out from the center of a wide (diameter $\approx$ 22 nm) barrel-shaped body labeled with 26 cholesterol on its membrane-facing side (Figure 5a). These cholesterol mediated tight binding of the nanopores to vesicles, as visualized by TEM, and with the help of voltage pulses, facilitated the pore insertion into suspended bilayers between two electrolyte-filled chambers, as confirmed by measuring electrical conductance of the

pore (~0.9 ns). Furthermore, the voltage-driven translocation of DNA hairpins and G-quadruplexes through the transmembrane channel was detected as current blockage events, whose amplitude and duration can be used as signatures to differentiate DNA species. Similar syringe-like designs were later adapted by the same group and Aksimentiev, Keyser et al. to build larger pores with channel widths up to 10 nm for faster molecular transport (Figure 5b).^[81–82] The large terminal area of the “syringes” provides ample space to graft plenty of hydrophobic moieties, which help the wide DNA channels to penetrate the bilayer. The bulkiness of the syringe body may also confer structural stability (at least on the cis-side of the membrane) to prevent pore collapse, and steric hindrance to deter irreversible pore aggregation in solution. However, such structures are often engineered using DNA-origami technique, meaning that they can be costly to build, considering the typical requirement of hundreds of oligonucleotides, long folding time (hours to days), and sometimes low assembly yield.

DNA nanopores with small channel widths (generally ~2 nm) can be built with simple geometries and a few DNA strands. In an extreme case, it took as few as one dsDNA molecule with six porphyrin labels to pierce through bilayers and allow conductance (~80 pS).^[83] Additionally, there is a large body of work describing needle-like transmembrane channels consisting of four to six bundled DNA duplexes carrying membrane anchors around them. In 2013, Howorka et al. reported the first example of such needle-shaped nanopores, featuring a six-helix bundle channel with up to 72 ethyl-modified, charge-neutral nucleotides forming a 2.2-nm wide hydrophobic belt around the outside (Figure 5c).^[84] Upon membrane incorporation, it exhibited conductance of ~0.4 nS, comparable to the measurement by Simmel and Dietz. In subsequent works, Howorka et al. modified the six-helix-bundle nanopore such that it can be closed via a lock strand that blocks the entrance of the cis-side of the channel, and re-opened by a key strand or elevated temperature that displaces the lock (Figure 5d).^[85–86] As expected, the pore in its closed state showed lower conductance than in its open state. Moreover, only after being unlocked did the pore allow a fluorescent molecule (sulforhodamine B) to cross a membrane and escape from a GUV. By contrast, 6-carboxyfluorescein, a more negatively charged fluorophore with similar molecular weight, remained in the GUV lumen in both the open and closed states of the pore, establishing the nanopore’s transport selectivity based on the charge of molecules.

Regardless of the shape and size of the nanopore, generating a DNA-made channel across a lipid bilayer is a process that consumes energy, which is compensated by embedding the hydrophobic moieties on the DNA channel into the bilayer. Based on a MD simulation by Howorka, Sansom et al., the entry of a six-helix bundle (outer diameter ≈ 6 nm) into a bilayer needs to overcome a barrier on the order of 10^2 kJ/mol, which makes it unsurprisingly the most energy demanding step in the entire process of pushing the channel through membrane.^[87] This requirement can be theoretically satisfied by the membrane incorporation of 2 or 3 cholesterol anchors (each releasing ~75 kJ/mol of free energy), which was corroborated by observations that 2–3 cholesterol were necessary to allow membrane penetration by such six-helix bundle nanopores. In an earlier study, Aksimentiev, Keyser, et al. stipulated ~1000 kJ/mol of energy for membrane insertion of a larger (outer diameter ≈ 11 nm) DNA channel.^[82] In this case, the energy was adequately compensated by the 19 cholesterol moieties at the end of the nanopore’s syringe-like body (Figure 5b). In

essence, these coarse-grained models mapped the energy landscapes for nanopore-bilayer interactions, thereby providing guidelines for designing DNA nanopores with thermodynamically favorable membrane incorporation.

Another important insight brought forth by the theoretical models is that the membrane anchors help with membrane insertion by priming the entry (i.e. putting the DNA structures in contact with the membrane) and preventing exit (i.e. setting a high dislodging barrier once embedded), instead of directly lowering the energy barrier of entry. Based on this, a reasonable hypothesis is that the DNA channels initially bind tangentially to the bilayer in search of an entry point and must reorient to a near-perpendicular position in order to puncture the membrane. While lacking direct evidence, this mechanism is supported by data showing that the membrane puncturing by a needle-like DNA nanopore requires multiple membrane anchors around the pore and that lipid-packing defects caused by high membrane curvature or external disturbance (e.g. detergent, electroporation) promote nanopore insertion.^[88] Therefore, arranging the hydrophobic moieties on the DNA structure in a way that favors its orientation change is important for efficient membrane puncturing. One illustrative example was reported by Howorka, Sleiman et al., which demonstrated the influence of cholesterol moiety number and arrangement on the DNA structure's membrane interactions.^[89] On a wireframe cube with 7-nm long dsDNA edges, when all eight vertices (or two vertices of each face) were decorated with cholesterol, the cubes inserted into GUV membranes and facilitated small molecule (Atto633) influx. In contrast, with four cholesterol labels on the same face of the cube, it could only adhere to the membrane without insertion (hence no Atto633 uptake), probably because such a cholesterol placement precludes the cube from reorienting on membrane.

Once a DNA channel opens a pore on a membrane, simulations show that lipids realign their headgroups with the DNA, allowing fast lipid exchange between bilayers. Keyser, Aksimentiev et al. found that the lipid transport rate between the inner and outer leaflets catalyzed by a four-helix-bundle nanopore exceeded 10^7 molecules per second, a whopping $\sim 1000\times$ higher than that achieved by biological enzymes (e.g. scramblases).^[90] Also of importance, a DNA channel situated in a lipid bilayer is subject to membrane pressure that can cause conductance fluctuation or even pore closure.^[91] As a result, the conductance of a DNA nanopore not only depends on its intrinsic properties, such as channel geometry, rigidity, and membrane anchor placement^[92], but is also affected by external factors such as ion strength^[87], voltage^[93], and membrane tension^[91] (Table 2). One thus needs to exercise caution when characterizing the nanopores by electrophysiology apparatuses or interpreting conductance data. Furthermore, it is worth noting that most DNA structures are porous due to the electrostatic repulsion between their constituent helices, which can cause current leakage.^[94] Possible solutions are hinted by recently developed crosslinking^[95] and coating^[96–97] methods that stabilize DNA nanostructures and the emerging DNA/protein hybrid pores^[98–99].

Interfacing DNA nanostructures with cell membranes

While researchers usually develop and test their membrane-manipulating DNA devices on model membranes (and rightfully so) a logical next step is to engineer nanostructures that

could interface with cells. Comparing to artificial lipid bilayers, cell membranes have more complicated chemical and physical properties. A cell typically displays leaflet asymmetry, a myriad of proteins, phase-separated domains, and ever-changing local curvatures, all of which are in a delicate balance under sophisticated regulation. This offers rich opportunities for designing nanostructures that recognize cell surface features (native or exogenous), elicit a response, and interfere with cell function. For example, using the SNARE-inspired DNA zippers, Fan et al. fused DNA-functionalized liposomes with DNA-tagged plasma membranes of cultured L1210 and HeLa cells for direct cytosolic delivery of liposome-encapsulated cytochrome c, which induced cell death.^[100]

Though most of the cutting-edge membrane-engineering techniques discussed in the previous sections have not been retooled for cellular applications, investigations on how to program and exploit DNA nanostructures' cellular interactions have been well under way. There have been a number of excellent reviews on this topic, for example an insightful overview of DNA nanotechnology's biological applications by Seelig et al.^[101], a comprehensive review of DNA-based drug delivery systems by Gu, Fan et al.^[102], and a focused discussion on cellular uptake of DNA nanostructures by Choi et al.^[103] In this section, we highlight several promising approaches to: (i) mediating cell-cell adhesion, (ii) regulating cell signaling, and (iii) modulating cell internalization.

Cell-cell adhesion plays many important roles in human physiology and development. Much like using DNA strands as molecular glues to mediate liposome association, modifying cell membranes with DNA can equip the cells with programmable adhesion handles. In addition to using membrane-bound DNA strands for building organoids^[104] and detecting molecular signals^[105], several pilot studies have utilized DNA nanostructures to program cellular interactions. For example, Liu, Chang, et al. designed multi-arm DNA structures bearing multivalent bispecific aptamers to engage Jurkat (a leukemia T cell line) and Ramos (a Burkitt's lymphoma B cell line) cells.^[106] More recently, Castro et al. built a DNA-origami plate with single-stranded overhangs on its top and bottom surfaces to selectively bind to different types of cells that are pre-tagged with cholesterol-modified DNA strands.^[107] Bridging the cell-membrane-displayed DNA plates with specific connecting strands led to homotypic or heterotypic cell-cell adhesion (Figure 6a). Though proving the concept, the DNA-nanostructure-mediated adhesions in these studies did not alter the cells' biological function.

Besides bringing cells into contact, clustering the right type/number of receptors could be the key to triggered cell signaling. This concept is exemplified by the work of Högberg, Teixeira et al., where a defined number of ephrin-A5 ligands were displayed at specific locations on a DNA-origami "nanocaliper" (Figure 6b).^[108] They found that clustered ephrin-A5 with certain spacing on the nanocalipers could upregulate the phosphorylation of EphA2 receptors on cultured human breast cancer cells, which resulted in the receptors' endocytosis and attenuated cell migration (i.e. decreased invasiveness). Moreover, dynamic DNA nanostructures present an opportunity to target receptors and stimulate signaling on specific cell types. Douglas, Bachelet, and Church encased antibodies within DNA-origami barrels locked by a combination of partially inhibited aptamers.^[109] Upon recognizing cell-type-specific biochemical markers (e.g. platelet-derived growth factors), the aptamers

underwent structural reconfiguration to unlock the barrels and present the antibodies for cell-surface receptor binding, thus triggering signaling (e.g. T-cell activation) in cultured cells. With a similar design, a team led by Zhao, Ding, Yan and Nie built a thrombin-loaded DNA tube locked by pre-inhibited aptamers specific to nucleolin (a cell-surface marker in tumor vessels) to selectively activate blood coagulation near tumors, leading to tumor necrosis in mice models.^[110] Another potentially powerful but so-far underexplored approach is to build mechanically tunable DNA structures for the study and manipulation of mechanosensitive receptors (e.g. channels, adhesion proteins) on cell membranes. A recent study by Ke, Salaita et al. mapped cell traction forces with surface-mounted DNA-origami rods modified with integrin-binding peptides and FRET-based tension sensors, thus moving this line of research to a new direction.^[111]

The well-orchestrated membrane dynamics can lead to the uptake, recycling, and degradation of objects on the cell surface. Therefore, establishing the internalization selectivity, efficiency, and pathway of membrane-bound DNA structures are important for applications that necessitate targeted subcellular delivery (e.g. genome editing). From a number of tissue-culture experiments that have each shed unique light on this topic^[112–117], the consensus seems to be the following. First, shape-defined DNA nanostructures generally enter cells more efficiently than unstructured oligonucleotides or dsDNA (e.g. plasmids). Second, geometry and molecular mass may affect DNA structures' cell uptake efficiency. Third, DNA structures labeled with lipophilic molecules or cell-specific ligands are more likely to be internalized compared to the unlabeled counterparts. Fourth, DNA structures can enter cells via multiple endocytic pathways, though a few studies highlighted the role of caveolin-dependent endocytosis. Fifth, the uptake profile of DNA structures could vary with different cell types. We note that, while these previous studies serve as valuable references for engineering DNA-based intracellular delivery platforms, they also suggest the importance of tailoring DNA structure design to specific applications (i.e. there is no one-size-fits-all solution). Additionally, the cell toxicity of artificial DNA structures needs to be experimentally evaluated. One example is that while labeling six-helix-bundle DNA rods with cholesterol moieties enhanced their uptake by HeLa cells without affecting the cell viability, labeling them with ethyl chains did lead to modest cytotoxicity (Figure 6c).^[118] In the future, a better understanding of DNA-nanostructure internalization, especially in regard to intracellular trafficking pathways, will be essential for the development of next-generation DNA-based reagents for theranostics (Figure 6d).^[119–120] Notably, Krishnan's group has used cargo-loaded DNA-icosahedron cages for cell-type-specific delivery of imaging probes and hormones *in vivo* (*C. elegans*), for mapping ligand-dependent endocytic pathways of DNA cages in live cells, and for spatiotemporally controlled release of hormones to stimulate cell signaling.^[121–124]

Future Directions

Interfacing DNA nanostructures with lipid-bilayer membranes adds to DNA nanotechnology's multifaceted applications in modifying, organizing, and shaping other materials (examples include metallic/semi-conducting nanoparticles, proteins, synthetic polymers, fluorophores, etc.). Like all other molecular engineering platforms powered by DNA nanotechnology, the precision and versatility of the lipid-interacting DNA devices

dictate the platform's membrane-manipulating capability. Hence, our endeavor to expand application and boost performance of membrane engineering tools will benefit from the fast evolving field of DNA nanotechnology, which constantly creates larger and more complex DNA structures, improves their assembly efficiency, and discovers new ways to control their motions. Nevertheless, the following challenges are present to nanoengineers hoping to make a DNA "Swiss army knife" to work on membranes. First, there is a need for a streamlined, intuitive-to-use software suite that simulates the behaviors of DNA nanostructures on lipid bilayers, providing molecular details and quantitative thermodynamic predictions. Given the complexity of the system, such modeling tools will likely have to be coarse-grained. A growing body of experimental and theoretical work has paved the way towards this goal, especially for modeling membrane-penetrating DNA pores and the freely-diffusing DNA structures on membrane. However, more systematic study on the biophysics of DNA-mediated membrane deformation is required to rationalize and reconcile current findings, and ultimately compile a comprehensive software package useful for guiding future designs. Second, despite a few recent attempts [58, 125], the field has yet to take full advantage of fast-developing microscopy tools (e.g. cryo-electron tomography, DNA PAINT^[126]) and chemical probes (e.g. tension sensors^[127]) to analyze the interplay between DNA structures and membranes with high resolution. Doing so will be especially useful in elucidating the behaviors of DNA devices deployed to biological membranes. Third, current DNA-enabled membrane-engineering tools are often associated with limitations that hinder their practical use, calling for experimental and design revamping of proof-of-concept prototypes. For instance, due to DNA and lipid misassembly, the yield of DNA-templated liposomes, especially those with complex curvatures, are insufficient for most ensemble biochemical assays. Economical ways to scale up the preparation, especially to enrich for correctly formed DNA-liposome complexes, is necessary to meet practical needs. Another example is that the negatively charged DNA structures, usually accompanied by cations (e.g. Mg^{2+}), may be incompatible with membranes of certain lipid and protein compositions. Possible solutions are to incorporate chemically-modified backbones (e.g. peptide nucleic acids) at the lipid-DNA interface or to shield the DNA surface with non-reactive polymers (e.g. polyethylene glycol). Fourth, comparing to the membrane-shaping protein machineries, our ability to manipulate membranes with DNA-based tools is rather rudimentary. Certain membrane remodeling events commonly found in cells, such as fission and invagination, are yet to be robustly recapitulated in model membranes using DNA nanostructures. The existing DNA devices lack the regulation mechanisms that control membrane deformation with high temporal resolution, such as the synchronized release of neural transmitters in response to calcium influx^[128]. Mimicking protein dynamics with reconfigurable DNA structures or grafting protein components onto DNA devices may lead to breakthroughs in this direction. Fifth, programming multiple synthetic devices to work cooperatively in organelle- or even cell-like systems remains a grand challenge. Achieving this goal will likely require a combination of bottom-up methods, such as DNA nanotechnology and protein engineering, and top-down approaches, such as microfluidics-based vesicle fabrication^[129–131], hence calling for the integration of multi-disciplinary design and fabrication approaches. Addressing these challenges will not only push the boundaries of basic research, including the quantitative study of membrane biophysics and the structure determination of membrane proteins, but also usher in numerous opportunities

in biotechnology, such as controllable, membrane-delimited nanoreactors, programmable cell-cell junctions (e.g. immune synapse), and possibly more efficient drug delivery vehicles.

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Figure 1. Self-assembled lipid and DNA structures and their bindings. (a) Schematic illustrations of common lipid structures. In clockwise order: a micelle, a nanodisc, a unilamellar liposome, a supported bilayer, and a multilamellar liposome. Inset shows an abstract model of a glycerol-phospholipid with two hydrocarbon chains. (b) A small collection of wireframe 3D shapes self-assembled from DNA. Inset shows a cartoon representation of a DNA double helix. (c) Binding between DNA and lipid-bilayer membranes can be mediated by electrostatic attraction (left), hydrophobic interaction (middle), and receptor-ligand binding (right).

Figure 2.

Behaviors of DNA structures bound to model membranes. (a) Distribution of cholesterol-labeled DNA rods in phase-separated lipid domains on GUV. Reproduced with permission from ref [16], copyright 2016, Cell Press. (b) Tracking the lateral diffusion of DNA-origami sheets on a supported bilayer by single-particle fluorescence microscopy. Reproduced with permission from ref [33], copyright 2014, ACS Publications. (c) A reconfigurable DNA sheet that self-folds via cholesterol anchors and opens upon membrane binding. Reproduced with permission from ref [39], copyright 2014, Wiley-VCH. (d) Photo-switchable dimerization of cholesterol-labeled DNA-origami hexagons on supported bilayers. Reproduced with permission from ref [46], copyright 2014, ACS Publications. (e) Self-assembling 2D arrays of unlabeled DNA-origami tiles via blunt ends on supported bilayer. Reproduced under the

terms of the CC-BY 4.0 license.^[47] Copyright 2015, Nature Research. (f) Blunt-end mediated self-assembly of unlabeled or cholesterol labeled three-point-star DNA tiles on mica and two different supported bilayers. Reproduced with permission from ref ^[28], copyright 2017, ACS Publications.

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Figure 3.

DNA-nanostructure-templated membrane structures. (a) Liposomes forming on the outside of a DNA-origami sphere. Reproduced with permission from ref [55]. Copyright 2014, ACS Publications. (b) Size-controlled liposomes forming on the inside of DNA-origami rings. Reproduced with permission from ref [56], copyright 2016, Nature Research. (c) An assortment of shape-defined liposomes forming inside DNA-origami cages. Reproduced with permission from ref [58], copyright 2017, Nature Research. (d) Generating size-controlled nanodiscs within DNA-origami barrels for the reconstitution of membrane proteins (left) and study of viral entry (right). Reproduced with permission from ref [61], copyright 2018, ACS Publications. (e) Protein-free nanodiscs formed inside DNA circles

densely modified with alkyl groups (two per turn). Reproduced with permission from ref [62], copyright 2018, RSC Publishing.

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Figure 4. DNA-guided membrane remodeling. (a) Schematics of membrane fusion between a supported bilayer displaying t-SNAREs and a DNA-templated liposome carrying a defined number of v-SNARE. Reproduced with permission from ref ^[57], copyright 2016, ACS Publications. (b) Liposome fusion and lipid tube bending driven by strand-displacement-mediated DNA-cage reconfiguration. Reproduced with permission from ref ^[58], copyright 2017, Nature Research. (c) Programmable GUV tubulation by curved DNA structures outfitted with cholesterol anchors. Reproduced under the terms of the Creative Commons CC-BY license.^[75] Copyright 2018, Nature Research.

Figure 5.

Transmembrane nanopores made of DNA. (a) A syringe-shaped DNA nanopore with a six-helix-bundle transmembrane channel and a cholesterol-decorated, barrel-shaped body. Reproduced with permission from ref ^[80], copyright 2012, AAAS. (b) A DNA nanopore similar to (a), but with a wider transmembrane channel. Reproduced under the terms of the Creative Commons CC-BY license.^[82] Copyright 2016, ACS Publications. (c) A needle-shaped DNA nanopore displaying a ring of ethyl groups. Reproduced with permission from ref ^[84], copyright 2013, ACS Publications. (d) A needle-shaped DNA nanopore with a displaceable ssDNA lock that controls the diffusion of small molecules. Reproduced with permission from ref ^[85], copyright 2016, Nature Research.

Figure 6. DNA nanostructure interacting with cell membranes. (a) DNA-nanostructure-mediated cell-cell adhesion. (b) DNA-nanostructure-induced cell signaling via binding of DNA-organized ligands to cell-surface receptors. (c) Induced cytotoxicity by puncturing cell membranes with DNA nanopores. (d) Concept of DNA nanostructures serving as target-specific drug delivery vehicles responsive to biological triggers.

Table 1.

Diffusion behaviors of selected membrane-anchored DNA nanostructures on GUV and supported lipid bilayers (SLBs).

DNA structure	Anchor type	No. of anchors	Diffusion coeff. $\mu\text{m}^2/\text{s}$	Model membrane	Ref
Hexagon of 10-bp edges	Porphyrin	1/ 2/ 3	2.0/ 0.9/ 0.6	SLB (DOPC)	[36]
$6 \times 6 \times 420 \text{ nm}^3$ rod (Figure 2a)	Cholesterol	8	1.39	GUV (DOPC)	[38]
$60 \times 90 \times 2 \text{ nm}^3$ sheet (Figure 2b)	Cholesterol	~47	0.71	SLB (DOPC:PEG-500-PE = 12.5:1)	[33]
$60 \times 35 \times 8 \text{ nm}^3$ brick	Cholesterol	4/ 8	0.4/ 0.26	SLB (DOPC:DiD = 99:1)	[29]
Dimer of the above brick		8/16	0.3/ 0.2		

Table 2.

Design and properties of membrane-spanning DNA nanopores.

DNA Structure	Pore size ^a	Channel length ^b	Membrane anchor	Max. No. of anchors	Tested voltage range (mV)	Conductance (nS) ^c	Molecule transport	Ref
Single duplex	N/A	5 nm	Tetraphenylporphyrin	6	± 100	0.08	N/A	[83]
Four-helix bundle	0.8 nm	11 nm	Cholesterol	4	± 100	>0.15	N/A	[72]
Six-helix bundle	2 nm	15 nm	Alkylphosphorothioates	72	± 100	0.395 ± 0.097	N/A	[84]
Six-helix bundle	2 nm	15 nm	Alkylphosphorothioates	72	50, 100, 150	0.6 (0.3 M KCl) 1.1 (1 M KCl)	N/A	[87]
Six-helix bundle (no lock)						1.62 ± 0.09		
Six-helix bundle (unlocked)	2 nm	9 nm	Cholesterol	3	± 100	1.34 ± 0.08	Sulforhodamine B	[85]
Six-helix bundle (locked)						0.66 ± 0.06		
Six-helix bundle	2 nm	14 nm	Tetraphenylporphyrin	2	Low (e.g. ±20) High (e.g. ±100)	1.62 ± 0.07 0.25 – 0.5	N/A	[93]
Six-helix bundle	2 nm	14 nm	Tetraphenylporphyrin	2	± 100	~ 0.25	N/A	[92]
Syringe shape with a six-helix-bundle channel	2 nm	47 nm	Cholesterol	26	± 200	0.87 ± 0.15	DNA hairpin and G-quadruplex	[80]
Syringe shape with a six-helix-bundle channel	2.6 nm	42 nm	Tocopherol (for plain bilayer) or biotin-streptavidin (for biotinylated bilayer)	26	75 – 125	1.6	N/A	[81]
Syringe shape with a six-helix-bundle channel	2.6 nm	28 nm		57		1.5	Atto 633	
T-shape with a 12-helix-bundle channel	3.7 × 4.2 nm ²	27 nm		57		3.1 ± 0.3	Atto 633, ssDNA, and dsDNA	
Syringe shape with a 16-helix-bundle channel	6 × 6 nm ²	54 nm	Cholesterol	19	± 100	30	N/A	[82]
Wireframe cube with double-stranded edges	7 × 7 nm ²	7 nm	Cholesterol	8	N/A	N/A	Atto 633	[89]

Notes:

^a, inner diameter or width by design;^b, including the transmembrane and extra-membrane domains;^c, mean value at 1 M KCl condition unless specified.

Table 3:

Acronyms of chemicals and proteins mentioned in this article

Full Name	Abbreviation
1,2-dioleoyl-sn-glycero-3-phosphocholine	DOPC
1,2-dipalmitoyl-sn-glycero-3-phosphocholine	DPPC
1,2-dioleoyl-sn-glycero-3-phospho-L-serine	DOPS
1,2-di-O-octadecenyl-3-trimethylammonium propane	DOTMA
1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]	PEG-2k-PE
1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-550]	PEG-500-PE
1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate	DiD
Soluble N-ethylmaleimide-sensitive factor attachment protein receptor	SNARE
Endosomal sorting complexes required for transport	ESCRT