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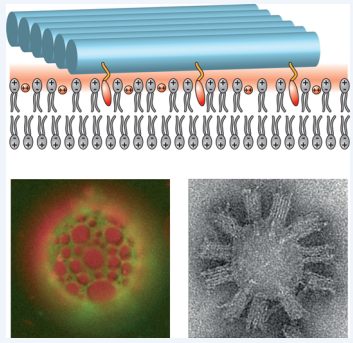
DNA Nanostructures Interacting with Lipid Bilayer Membranes

Martin Langecker,[†] Vera Arnaut,[†] Jonathan List,[†] and Friedrich C. Simmel*[,†,‡]

[†]Physics Department − E14 and ZNN/WSI, Technische Universität München, Am Coulombwall 4a, 85748 Garching, Germany

[‡]Nanosystems Initiative Munich, Schellingstr. 4, 80539 Munich, Germany

CONSPECTUS: DNA has been previously shown to be useful as a material for the fabrication of static nanoscale objects, and also for the realization of dynamic molecular devices and machines. In many cases, nucleic acid assemblies directly mimic biological structures, for example, cytoskeletal filaments, enzyme scaffolds, or molecular motors, and many of the applications envisioned for such structures involve the study or imitation of biological processes, and even the interaction with living cells and organisms.



An essential feature of biological systems is their elaborate structural organization and compartmentalization, and this most often involves membranous structures that are formed by dynamic assemblies of lipid molecules. Imitation of or interaction with biological systems using the tools of DNA nanotechnology thus ultimately and necessarily also involves interactions with lipid membrane structures, and thus the creation of DNA−lipid hybrid assemblies. Due to their differing chemical nature, however, highly charged nucleic acids and amphiphilic lipids do not seem the best match for the construction of such systems, and in fact they are rarely found in nature. In recent years, however, a large variety of lipid-interacting DNA conjugates were developed, which are now increasingly being applied also for the realization of DNA nanostructures interacting with lipid bilayer membranes. In this Account, we will present the current state of this emerging class of nanosystems. After a brief overview of the basic biophysical and biochemical properties of lipids and lipid bilayer membranes, we will discuss how DNA molecules can interact with lipid membranes through electrostatic interactions or via covalent modification with hydrophobic moieties. We will then show how such DNA−lipid interactions have been utilized for the realization of DNA nanostructures attached to or embedded within lipid bilayer membranes. Under certain conditions, DNA nanostructures remain mobile on membranes and can dynamically associate into higher order complexes. Hydrophobic modification of DNA nanostructures can further result in intra- or intermolecular aggregation, which can also be utilized as a structural switching mechanism. Appropriate design and chemical modification even allows insertion of DNA nanostructures into lipid bilayer membranes, resulting in artificial ion channel mimics made from DNA. Interactions of DNA nanodevices with living cells also involve interactions with membrane structures. DNA-based nanostructures can be directed to cell surfaces via antibody−antigen interactions, and their cellular uptake can be stimulated by modification with appropriate receptor ligands. In the future, membrane-embedded DNA nanostructures are expected to find application in diverse areas ranging from basic biological research over nanotechnology to synthetic biology.

1. INTRODUCTION

DNA nanotechnology has experienced an enormous development over the past decades.[1−3] As discussed also by other Accounts in this special issue of *Accounts of Chemical Research*, today almost arbitrarily shaped nanoscale molecular structures can be realized using the unique, sequence-specific self-assembly properties of DNA. By chemical functionalization, DNA nanostructures can be further utilized as rigid molecular scaffolds for the precise positioning of molecules or other nanoscale objects.

In biology, structural control and precision are usually provided by proteins, and in fact many DNA nanostructures have been previously realized that essentially imitate protein structures: cytoskeletal filaments, molecular cages, or enzyme scaffolds. Our ability to generate DNA nanostructures much more easily than proteins currently comes at the price of a reduced chemical richness and typically lower functionality, but this might in the future be compensated by the use of synthetic nucleic acid bases and analogues. However, even if we *could* substitute proteins by DNA assemblies, important features for a bioinspired nanotechnology would still be missing, in particular large scale spatial organization and compartmentalization.

An essential class of biomolecules involved in biological compartmentalization are the lipids. Superstructures formed by their self-assembly—micelles, vesicles, and membranes—are of a different nature than the structures formed by proteins or nucleic acids. Based on hydrophobic interactions, lipid−lipid interactions are less "precise" and more dynamic, but at the same time capable of forming large and extended molecular assemblies. In biology, membranes separate functionally distinct regions from each other, while membrane-embedded molecules are used for intercompartmental communication and transport. Organization of enzymes and other functional molecules within membranes provides a reduction in dimensionality and allows them to interact more effectively than in three dimensions.

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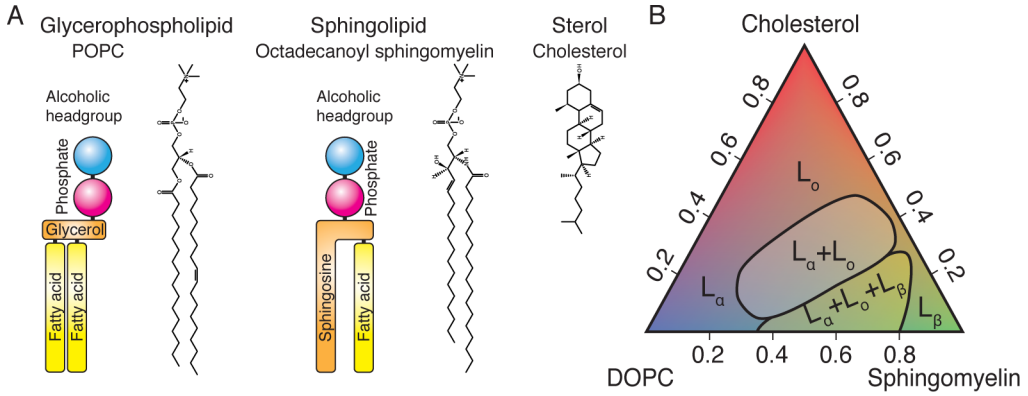

Figure 1. Lipids. (A) Structures of lipid types. Glycerophospholipids are composed of glycerol attached to a polar headgroup (a phosphate esterized with an alcohol) and two fatty acid chains. The molecular structure shown is POPC. Sphingolipids are based on sphingosine, exemplified here by octadecanoyl (18:0) SM. Cholesterol is the most common sterol. (B) Phase diagram of a mixture of DOPC, sphingomyelin, and cholesterol. For certain compositions, two phases (liquid ordered L_o and liquid crystalline L_α) coexist. At low cholesterol concentrations, a third phase (solid-ordered L_β) is also found. Adapted with permission from ref 4. Copyright 2005 American Physical Society.

Based on the recent advances in structural DNA nanotechnology, it has now become possible to construct hybrid systems composed of DNA nanostructures and lipids and, thus, harness the peculiar physicochemical features of membrane assemblies, which are of enormous interest for a variety of applications ranging from basic biological research to nanotechnology. In the following, we will review the biophysics and biochemistry of lipid bilayer membranes and their interactions with nucleic acids. We will discuss the recent development of DNA nanoassemblies attached to or embedded within lipid membranes, and also speculate on future directions for this emerging class of DNA nanostructures.

2. LIPID BILAYER MEMBRANES

2.1. Lipids and Their Assemblies

Lipids are small organic molecules that are soluble in nonpolar solvents, but typically insoluble in water. The lipids most abundantly found in nature are glycerophospholipids, sphingophospholipids and sterols (Figure 1A). Phospholipids are composed of a hydrophobic hydrocarbon chain region (with different chain patterns), a linker molecule (such as glycerol) and a hydrophilic headgroup containing a phosphate group. The headgroup may be charged or zwitterionic neutral. The most important natural sterol is cholesterol, which alone does not form membranes, but interacts strongly with other lipids, most preferentially with sphingolipids.[4] The structural organization of lipids in water results from a compromise between hydrophobic association, steric hindrance and ionic repulsive forces. Depending on their size and shape, lipids can self-associate to form micelles (Figure 2A) or bilayer structures (Figure 2B). Hydration of dried lipid films leads to the formation of small and large unilamellar vesicles (SUVs and LUVs) with typical sizes of 25−100 nm and ≈1 μm. Additional application of electrical fields can result in the formation of cell-sized giant unilamellar vesicles (GUVs) (Figure 2C). The curvature of lipid membranes (Figure 2D) plays an important role in the organization and compartmentalization of cells and cell organelles, and changes in membrane curvature and topology are involved in many essential biological processes such as budding and fusion of vesicles, or cell division.[5] An asymmetry in lipid distribution and the (passive) presence of membrane proteins can induce a spontaneous curvature, but large membrane deformations require substantial forces that have to be supplied by specialized membrane-sculpting proteins.[6]

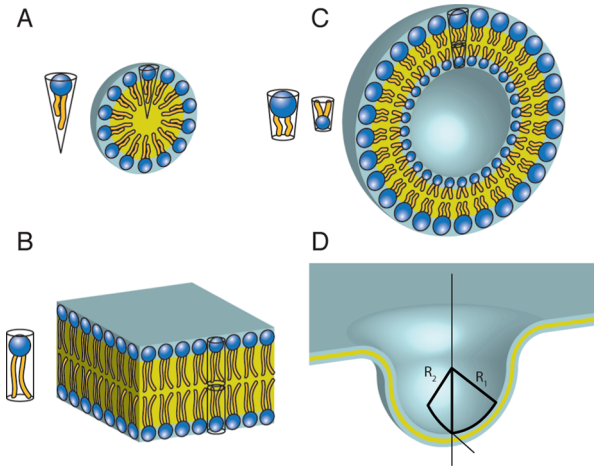

Figure 2. Lipid assemblies. (A) Micelles, preferentially formed by lipids with an inverted conical shape. (B) Planar lipid bilayers, formed by lipids with a more cylindrical shape. (C) Vesicles are spherical lipid bilayers with a water core. (D) Schematic protrusion in a planar lipid bilayer with corre sponding radii of curvature.

2.2. Lipid Membrane Phases

Lipid bilayer membranes display a rich phase behavior. At low temperatures, membranes are in a solid-ordered gel (L_β) phase, whereas at higher temperatures a liquid-disordered (liquid-crystalline) phase (L_α) is adopted. The corresponding phase transition temperature depends on hydrocarbon chain length and lipid headgroup. In mixtures of lipids, several phases may exist simultaneously. Mixtures of cholesterol and phospholipids may obtain a liquid-ordered (L_o) phase that is distinct from the L_β phase and is not found in the absence of cholesterol.[7] In this context, extensively studied model systems are three-lipid mixtures containing cholesterol, phosphatidylcholine (PC) lipids and sphingomyelin (SM). For instance, mixtures of DPPC/DOPC/chol[8] or SM/DOPC/chol (Figure 1C)[4] show several regions of two-phase and one region of three-phase coexistence. Cholesterol is predominantly found in the L_o phase, which is rich in DPPC or SM, respectively. The phase properties of membranes are assumed to play a key role in

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biological membrane function. It is known that the majority of membrane-associated proteins prefers liquid over solid phases, with the exception of proteins that have a precise hydrophobic matching with the solid state. For coexistent liquid-ordered and liquid-disordered states, most membrane proteins partition into the disordered phase.

3. INTERACTIONS BETWEEN DNA AND LIPID BILAYER MEMBRANES

3.1. Electrostatic Interactions

As a negatively charged polyelectrolyte, DNA strongly interacts electrostatically with cationic lipids such as DOTAP or DOTMA (Figure 3A). In their presence, DNA is compacted and forms spherical "lipoplexes".[9] The interaction is strongest for low salt concentrations, when electrostatic screening effects are small.[10]

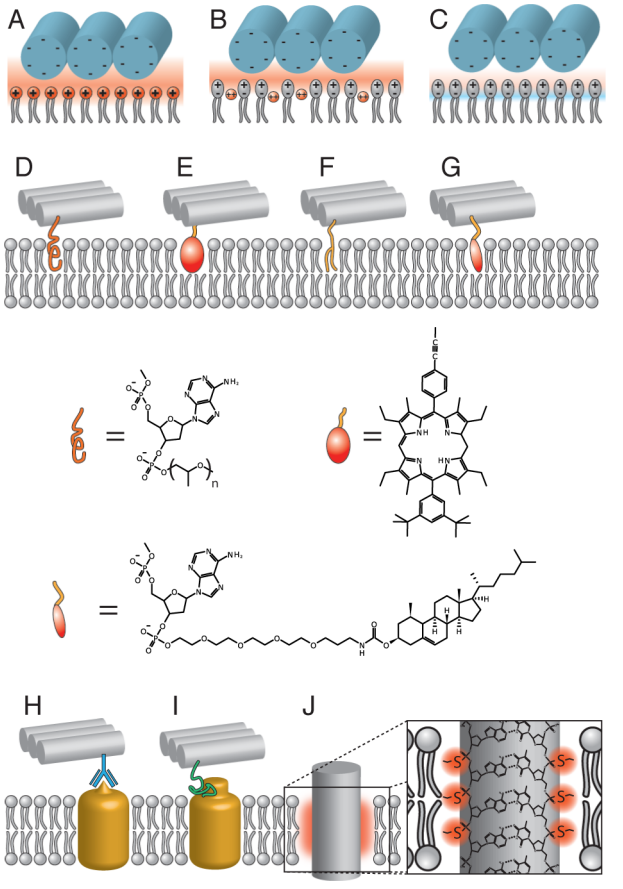

Figure 3. DNA–lipid interactions. (A–C) Electrostatic binding to (A) cationic lipids, (B) zwitterionic lipids in the presence and (C) in the absence divalent cations. (D–G) Amphiphilic DNA conjugates with (D) PPO, (E) porphyrin, (F) lipid, (G) cholesterol. (H, I) Interactions involving membrane-associated proteins: (H) DNA–antibody conjugates and (I) aptamer–protein interaction. (J) Hydrophobic ethyl-thiophosphate backbone modification.

In the presence of divalent cations such as Mg^{2+} or Ca^{2+}, DNA also binds strongly to zwitterionic lipids,[11,12] but the mechanism of the interaction is not fully understood. While for anionic lipids divalent cations are assumed to bridge negatively charged lipid headgroups and the DNA backbone, for zwitterionic lipids the cations are thought to insert between the phosphates of neighboring lipid molecules. This neutralizes the negative charge on each lipid, resulting in a net positive charge (see Figure 3B).[11,12] Some studies report weak binding to zwitterionic lipids also in the absence of divalent cations (Figure 3C),[13,14] potentially mediated by ion–dipole interactions.[15]

Electrostatic binding is affected by lipid composition and phase behavior. Experimentally, dsDNA was observed to preferentially bind to the L_o phase,[14,16] which may be explained by the closer packing and thus higher surface charge density of the lipids.

3.2. Hydrophobic Functionalization

DNA-based nanostructures can also be attached to lipid membranes using DNA conjugates with lipids or other hydrophobic molecules (see Figure 3D–G). DNA-lipid conjugates are used for gene transfection, they can be used to induce vesicle fusion[17,18] or to specifically cross-link vesicles or even cells.[19−21] Lipid-DNA conjugates can be generated by incorporation of lipids during DNA synthesis using phosphoramidite coupling, or postsynthetically by conjugation to thiol- or amino-modified DNA oligonucleotides. The conjugation step can be performed directly on a lipid membrane containing a small fraction of reactive lipids.[22]

Cholesterol is probably the most commonly used lipid for DNA conjugation, although its association with membranes is comparatively weak. This can be compensated by the use of multiple cholesterol modifications, however, and already a combination of two cholesterols was observed to lead to almost irreversible binding to membranes.[23]

The binding properties of cholesterol are strongly affected by the nature of the linker through which it is attached to DNA. Cholesterol alone influences lipid membrane packing and increases its order.[24] In contrast, cholesterol linked to tetraethylene glycol (TEG) was not observed to alter membrane structure. In DLPC:DPPC:chol mixtures with coexistent solid and fluid phases, chol(-TEG)–DNA conjugates were observed to partition into the liquid disordered phase (L_α) only.[25] In mixtures containing two fluid phases such as DOPC:DPPC:chol, chol(-TEG)–DNA conjugates distributed evenly into both phases, while cholesterol alone is known to strongly prefer the L_o-phase. Bivalent conjugates were observed to prefer the L_o over the L_α phase for this lipid mixture.

Apart from lipids, other hydrophobic molecules can be attached to DNA. For example, DNA has been conjugated to α-tocopherol,[26] poly(propylene oxide) (PPO),[27] or porphyrin[28,29] (see Figure 3F), and were shown to incorporate into lipid membranes. Finally, the DNA backbone itself may be alkylated to become hydrophobic[30] (see Figure 3G).

3.3. Interactions Involving Membrane-Bound Proteins

DNA may also be coupled to membranes via membrane-associated molecules. These can be highly specific for a certain cell type, or even for the disease state of a cell, and thus can be utilized for targeting biological membranes more specifically. Binding to membrane proteins has been achieved using aptamer–target interactions,[31,32] DNA-antibody conjugates,[33,34] or by direct covalent coupling.[35] Such interactions do not, however, necessarily involve a direct interaction with the lipid membrane itself.

4. INTERACTIONS BETWEEN DNA-NANOSTRUCTURES AND LIPID BILAYER MEMBRANES

Since the initiation of structural DNA nanotechnology by Nadrian Seeman in the early 1980s, DNA has been shown to be

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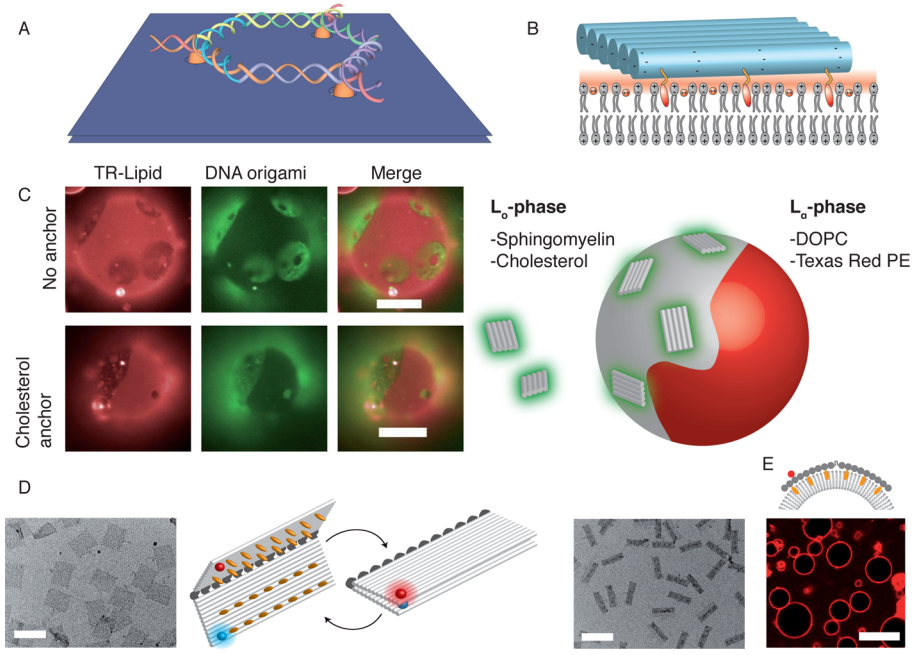

Figure 4. DNA nanostructures on lipid bilayer membranes. (A) A hexagonal DNA nanostructure attached to a lipid bilayer membrane using porphyrin anchors.[28] (B) Interplay of hydrophobic and electrostatic interactions upon DNA nanostructure binding. (C) DNA origami structures on phase-separated vesicles. DNA rectangles of dimension 90 nm × 65 nm and labeled with YOYO-1 (green) are bound to vesicles with (bottom row) and without (top row) cholesterol modification in the presence of 12.5 mM Mg^{2+}. Lipid vesicles were made from a (2:2:1:0.01) mixture of (SM:DOPC:Chol:Texas Red PE). Fluorescently labeled PE lipids (red) partition into the DOPC-rich L_α phase, while DNA origami structures are bound to the L_o phase under these conditions. Scale bars are 25 μm. (D) Strong hydrophobic interactions between 28 cholesterol modifications can fold a single-layered DNA origami structure into a DNA bilayer (TEM image right). The structure can be unfolded by the addition of a surfactant (TEM on the left). (E) The bilayer structure also unfolds in the presence of lipid vesicles and binds to lipid bilayer membranes. Shown is a confocal fluorescence image of origami covered GUVs. Panels (D) and (E) are adapted from ref 45.

an extremely versatile material for the assembly of molecular objects,[36,37] extended two-dimensional lattices,[38] and molecular crystals.[39] DNA origami[40,41] and related techniques[42] based on the association of a large number of oligonucleotides today allow the realization of almost arbitrarily shaped molecular objects on a length scale of 10−100 nm. As an emerging theme in this field, researchers have recently started to study the interactions of such DNA nanostructures with lipid bilayer membranes.

4.1. Attachment of DNA Nanostructures to Lipid Bilayer Membranes

As discussed above, DNA nanostructures may be bound electrostatically to lipid bilayer membranes or by functionalization with membrane-interacting molecules. One of the first examples of a DNA-based nanostructure interacting with a lipid bilayer membrane was a porphyrin-functionalized DNA-based hexagon (Figure 4A).[28] The hexagon was constructed from six oligonucleotides and had a side-length of 10 basepairs, with 39 nt long single-stranded extensions at three of its corners. To these extensions, porphyrin-functionalized oligonucleotides[29] were hybridized, which facilitated attachment of the hexagons to DOPC liposomes with diameters of ≈100 nm. The orientation of the DNA nanostructures on the lipid bilayer could be controlled by the number and position of the porphyrin anchors.[43]

Our own group studied the binding of single-layered DNA origami structures to supported lipid bilayer membranes and GUVs (Figure 4B). In the presence of Mg^{2+} ions, unmodified rectangular DNA origami structures bound electrostatically to zwitterionic PC lipid bilayers. Large concentrations of monovalent ions promoted unbinding of the structures. Stronger attachment, even at high monovalent salt, was achieved by functionalization of the origami structures with cholesterol anchors. In phase-separated membranes made from lipid mixtures and in the presence of Mg^{2+}, the origami structures preferentially bound to the liquid ordered phase (Figure 4C).

Similar studies were performed by Czogalla et al.[44] using origami six-helix bundle (6HB) structures of length 420 nm and diameter 6 nm. In the absence of divalent ions, cholesterol-TEG-functionalized 6HBs were found to preferentially bind to the disordered phase of phase separated GUVs, while upon addition of Mg^{2+} the helix bundles migrated into the ordered phase. This process could be reversed by chelation of magnesium ions with EDTA. Structures with and without cholesterol modifications also bound strongly to bilayers containing cationic DOTAP, while only cholesterol-modified structures were observed to bind to bilayers with negative DOPS, and only at high ionic strength.[44]

Recently, we found that the hydrophobic interactions between cholesterol molecules can be utilized to bend and fold DNA nanostructures with multiple cholesterol modifications (Figure 4D).[45] We utilized this effect to create a switchable DNA bilayer structure that unfolded after the addition of surfactants, or in the presence of lipid bilayer membranes (Figure 4E). The switching principle could also be combined with a key-lock mechanism responding to specific molecular signals. Membrane attachment of DNA nanostructures can thus be made context-dependent.

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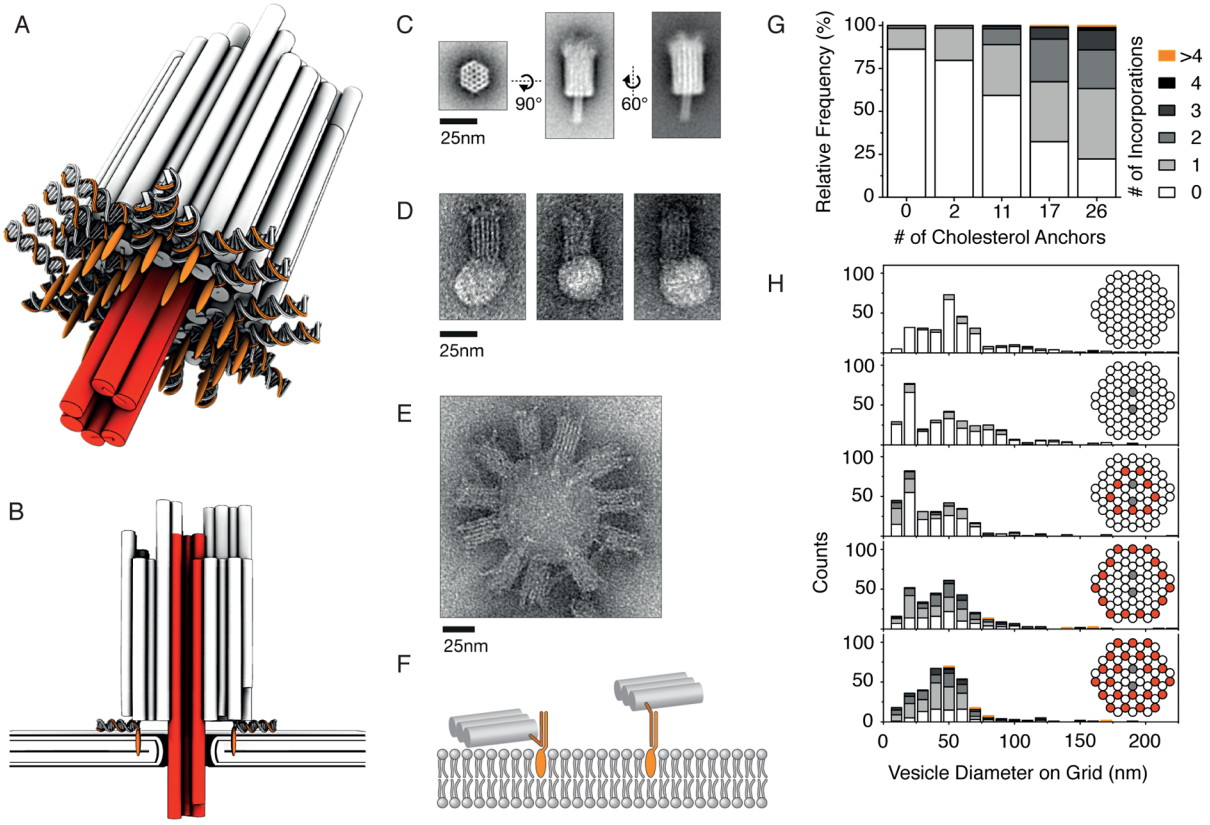

Figure 5. DNA-based membrane channel.[47] (A) Schematic design of the channel consisting of a barrel-shaped cap (white) and a transmembrane stem (red). Interaction with lipid membranes is mediated by cholesterol modifications (orange ellipsoids). (B) Cross-sectional view of a membrane-incorporated channel structure. (C) Class-averaged TEM images from purified channel structures. (D, E) Representative TEM images of DNA channels adhering to small unilamellar vesicles (SUVs) made from POPC. (F) Alternative geometries for the attachment of cholesterol-modified adaptor strands. (G) Binding efficiency of DNA channels to SUVs as a function of the number of cholesterol anchors. (H) Binding efficiency as a function of size of the vesicles for various numbers of cholesterols and different attachment positions. Red circles, cholesterol at the bottom of the cap; gray circles, cholesterols at the side of the six-helix stem.

4.2. Diffusion of DNA Nanostructures on Lipid Bilayers

Fluid lipid bilayer membranes differ considerably from conventional solid supports for DNA-based nanostructures. Lipid membranes may be used as soft substrates for molecular self-assembly, on which DNA-based nanostructures can dynamically assemble and disassemble similarly to membrane-bound protein complexes in biology. Conversely, it is also conceivable to influence membrane structure by DNA nanostructures that mimic membrane-sculpting proteins.

In this context, the mobility of DNA nanostructures on lipid bilayers is important: are they pinned to the membrane, do they diffuse with their lipid anchors, or do they form rafts? Diffusion coefficients of lipids within supported bilayer membranes were studied using fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy (FCS), and other techniques. In the liquid crystalline membrane phase, they are typically on the order of 1−10 $\mu m^2/s$ at room temperature.

FRAP experiments performed on the small DNA hexagons of the previous section attached to supported lipid bilayers resulted in diffusion coefficients of 0.6, 0.9, and 2.0 $\mu m^2/s$, for 3, 2, and 1 porphyrin anchors, respectively.[28] Also the mobility of cholesterol-TEG labeled DNA 6HBs on the surface of GUVs was studied using FRAP and FCS.[44] At high DNA origami densities, diffusion was strongly inhibited. At lower densities, the translational and rotational diffusion coefficients were determined to be 1.4 $\mu m^2\ s^{-1}$ and 68 $rad^2\ s^{-1}$, respectively. The mobility is affected by the number and position of the hydrophobic anchors, and the presence of divalent ions.

Suzuki et al. demonstrated the first assembly/disassembly reaction of a DNA nanostructure on a supported SM/DOPC lipid bilayer.[46] In this study, DNA origami-based hexagons were dimerized using photoswitchable azobenzene linker oligonucleotides. Hexagon dimers were bound to the lipid bilayer using cholesterol-TEG modifications, which preferred the SM rich, liquid ordered state of the membrane. Upon irradiation with light, the dimers disassembled into monomers, which could be directly monitored using high-speed AFM imaging. The monomers could diffuse on the bilayer and reassemble after photoswitching the azobenzene linkers back into their hybridization competent state.

4.3. Artificial Membrane Channels Made from DNA

With the recent advances of DNA nanotechnology, it has become possible to realize DNA nanostructures that mimic the functions of proteins, and the imitation of membrane-associated proteins is particularly promising in this context. Membrane-bound DNA structures could be used for the study of processes in membrane biology, they could constitute components of cell-scale bioreactors, or they could be utilized as membrane-bound biosensors.

The realization of artificial membrane-penetrating pores is challenging, however. The energetic cost associated with the

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insertion of DNA into the hydrophobic core of a lipid membrane is prohibitively high. Considering the difference in electrostatic energy in water and in the membrane, already the insertion of a single monovalent ion would cost several 100 kJ/mol!

We could recently demonstrate, however, that by appropriate hydrophobic functionalization it is in fact possible to achieve stable incorporation of DNA nanostructures into lipid bilayer membranes. Using scaffolded DNA origami, we constructed the artificial membrane channel structure shown in Figure 5A,C.[47] The structure consisted of 54 parallel DNA double helices, of which the central six were designed as a transmembrane channel. The bottom of the cap structure was functionalized with cholesterol in order to anchor the structure in the membrane (Figure 5B). For the formation of such a structure, the lipids have to arrange around the DNA channel, resulting in a hydrophilic pore around its stem. Up to linear order the formation of a small hole of radius R in a bilayer membrane requires the energy $E \approx 2\pi\gamma_L R$, where γ_L is the line tension of the membrane.[48] From this one can estimate that the accommodation of a 6HB of diameter 6 nm costs on the order of ≈50−500 kJ/mol.[47] The "hole formation energy" is in fact easily compensated by the free energy gained by binding of the cholesterols to the lipid membrane and by electrostatic interactions mediated by Mg^{2+} ions.[47] The main obstacle for membrane penetration is the activation of this process, which can be facilitated by membrane defects or electrical fields.

TEM studies on SUVs confirmed that the channels indeed bound to lipid membranes (see Figure 5D,E). As expected, the number of cholesterols strongly influenced the binding efficiency (see Figure 5G,H), and the incorporation probability increased for small vesicle diameters. Ultimate proof for channel formation was provided by electrical measurements. The incorporation of membrane channels was observed as a stepwise increase in transmembrane current, with a conductance in the nanosiemens range. Electrically stable insertions were only achieved when the channel was anchored via cholesterols proximal to the cap structure (Figure 5F).

Unlike most biological membrane channels, the structure of Figure 5A does not contain a hydrophobic ring matching the hydrophobic core of the membrane, which results in the formation of a hydrophilic pore as discussed above. Recently, a short DNA 6HB nanopore structure was realized that was supplied with a hydrophobic ring made from either ethylthiophosphate[49] or porphyrin groups,[50] and was also shown to successfully penetrate lipid bilayer membranes in electrical recordings.

5. INTERACTIONS BETWEEN DNA NANOSTRUCTURES AND CELLS

Nanomedicine is seen as a promising area of application for DNA nanostructures. One of the visions of this field is the realization of drug delivery and controlled release systems as sketched in Figure 6A. The delivery and release process involves interactions with many biological membrane structures—the cell membrane itself, but potentially also intracellular membranes and compartments.

So far, there have been mainly phenomenological studies on cellular uptake and intracellular stability of DNA-based nanostructures. They were found to maintain their structural integrity when incubated in cell lysate,[51] and they were found nontoxic and stable to enzymatic degradation inside of cells.[52] Uptake was studied in various mammalian cell lines using DNA

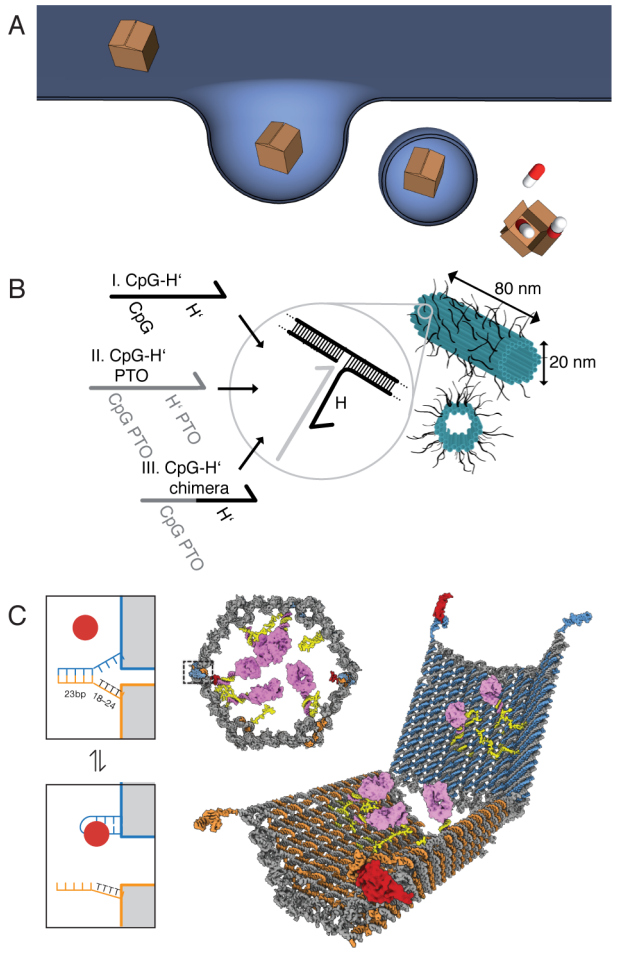

Figure 6. Interactions with cells. (A) Illustration of drug delivery via endocytosis using DNA-based nanocontainers (schematically represented by a "box"). (B) Cytosine-phosphate-guanine (CpG)-modified DNA origami tubes successfully used for immunostimulation. Adapted with permission from ref 54. Copyright 2011 American Chemical Society. (C) DNA-based nanodevice, specifically delivering molecular payloads to a cell surface using an aptamer based membrane recognition mechanism (insets). Reprinted with permission from ref 34. Copyright 2012 AAAS.

tetrahedra,[52,53] triangles and tubes,[54−56] typically using fluorescence microscopy or FRET. DNA nanostructures are assumed to enter the cell via endocytosis (Figure 6A). For small structures like tetrahedra with a size of ≈7 nm improved uptake was observed when complexed with the transfection agent Lipofectin,[52] a 1:1 mixture of the cationic lipid DOTMA and the zwitterionic lipid DOPE. Surprisingly, for a larger structure with dimensions 20 nm × 80 nm uptake was superior for structures in the absence of transfection agent (Figure 6B).[54] In general, cell uptake seems to depend on size, compactness and shape of the carriers.[54,56] The localization of DNA nanostructures inside cells is still controversial. While most studies indicate that the structures did not escape the endosome and ended up in the lysosome after ≈12h, others claim localization in the cytoplasm.

In some applications, cell uptake may not be required, but rather cell-specific targeting and attachment to a cell membrane. An example for an extracellular cargo-release system developed by Douglas et al.[33] is shown in Figure 6C. The system consisted of a tubular DNA container, whose two halves were connected by a hinge on one side and closed by

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molecular "locks" on the other. The locks were composed of DNA aptamers hybridized to complementary sequences. In the presence of antigen "keys", the aptamers preferentially bound to these, unlocking the barrel and thus revealing its molecular payload. Apart from antibody–antigen interactions with cell surface epitopes, other binding strategies can be utilized. For instance, hydrophobic cholesterol and dialkylphosphoglyceride have been shown to interact efficiently with cell membranes and can be easily conjugated to DNA.[20,21] Single-stranded DNA spacers can be used to penetrate the dense glycocalix on the cell surface and thus reach into the cell's lipid bilayer membrane.

An amazing achievement was the recent creation of virus-inspired, membrane-encapsulated octahedral DNA origami structures by Perrault and Shih.[57] In their approach, DNA–lipid conjugates were first hybridized to the DNA nanostructures in the presence of a surfactant. After the addition of liposomes and subsequent dialysis, lipid bilayer membranes formed around the nano-octahedra. Encapsulation in PEGylated membranes resulted in strongly improved nuclease-resistance and a considerable reduction of immune activation in vivo.

6. FUTURE PERSPECTIVE

Hybrid assemblies made from DNA nanostructures and lipid bilayer membranes have a huge potential for applications in various fields, ranging from nanotechnology over basic biological research to synthetic biology and nanomedicine.

In nanotechnology, membranous structures could be used to direct self-assembly reactions, for example, as soft two-dimensional supports for polymerization of DNA tectons (Figure 7A). Such platforms might also facilitate the dynamic assembly and disassembly of nonequilibrium structures similar to, for example, cytoskeletal networks found in biology. DNA nanostructures might also be used as rigid scaffolds for membrane structures and improve their mechanical stability.

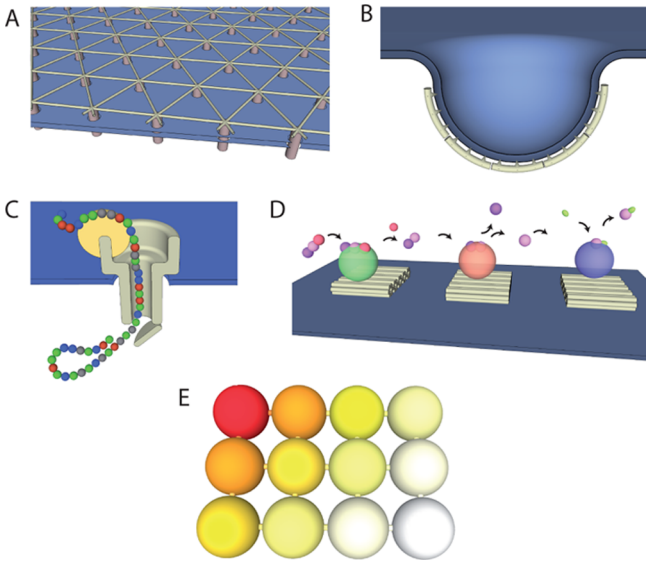

Figure 7. Future perspectives. (A) Lipid bilayers as soft scaffolds for the assembly of DNA-based supramolecular networks. (B) Membrane-sculpting DNA nanostructures. (C) Voltage and ligand-gated synthetic membrane channels. (D) Membrane–based reaction cascades. (E) Communicating networks of cell-scale reaction compartments.

DNA mimics of naturally occurring membrane-shaping proteins such as clathrin, dynamin, BAR, or SNARE proteins[6] could be used to bend and shape lipid bilayer membranes, induce vesicle budding or fusion (Figure 7B). Such capabilities might also prove useful for improving the efficiency of DNA-based delivery systems. DNA membrane channels such as those discussed in section 4 could be further developed into versatile membrane biosensors that can sense single molecule binding and translocation events or transmembrane voltage analogous to naturally occurring ion channels (Figure 7C). DNA channels might also act as molecular sieves or pumps, and integration of several functions in multisubunit complexes is easily conceivable.

Finally, membrane-bound DNA nanostructures are promising components of artificial cell-scale reaction compartments. Localization of chemical reactions and cascades on membrane-bound DNA nanostructures might benefit from compartmentalization and confinement similar to biochemical reactions in cells (Figure 7D). One could even think of the assembly of artificial "tissues" using structural DNA components, in which specialized vesicle reactors communicate and exchange materials in a coordinated manner (Figure 7E).

AUTHOR INFORMATION

Corresponding Author

*E-mail: simmel@tum.de.

Notes

The authors declare no competing financial interest.

Biographies

Martin Langecker is a graduate student at the Physics Department of the TU-München. He is currently working on single molecule nanopore experiments.

Vera Arnaut is a graduate student at the Physics Department of TU München. She is currently working on single molecule nanopore experiments.

Jonathan List is a graduate student at the Physics Department of TU München. His area of research is DNA nanotechnology.

Friedrich C. Simmel obtained his Ph.D. in physics 1999 at LMU München, and currently is a professor of physics at TU München. His research is devoted to the realization of synthetic biomolecular structures and systems.

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ABBREVIATIONS

DPPC - 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine
POPC - 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine
DOPC - 1,2-dioleoyl-*sn*-glycero-3-phosphocholine
DLPC - 1,2-dilauroyl-*sn*-glycero-3-phosphocholine
DOPE - 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine
DOPS - 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine
DOTAP - 1,2-dioleoyl-3-trimethylammonium-propane
DOTMA - 1,2-di-*O*-octadecenyl-3-trimethylammonium propane
SM - *N*-octadecanoyl-D-erythro-sphingosylphosphorylcholine

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