Mimicking Membrane-Related Biological Events by DNA Origami Nanotechnology

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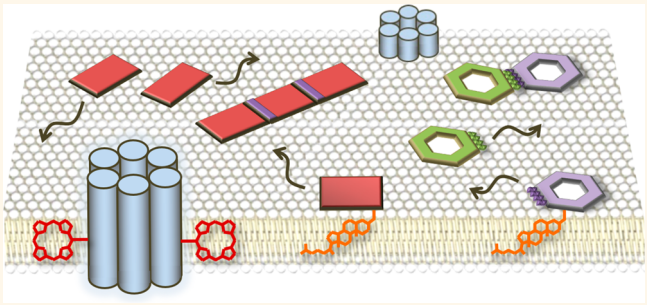
ABSTRACT One of the potential applications of DNA nanotechnology is the construction of two- or three-dimensional nanostructures that mimic the function of existing biological molecules. In this issue of *ACS Nano*, Kocabey *et al.* demonstrate that lipid-bilayer-anchored DNA origami structures can be assembled into prescribed superstructures in a programmed manner. The reported DNA-based artificial system can mimic the dynamic assembly of membrane-associated protein clusters that play an essential role in deformation of cellular membranes.



Membrane proteins and membrane-associated proteins mediate various fundamental biological processes including molecular transport, signal transduction, energy conversion, and intercellular communication. Dysfunction of these proteins can cause severe diseases and can be lethal. It is therefore not surprising that structure–function relationships of membrane proteins and membrane-associated proteins have been of great interest in diverse fields ranging from basic cell biology to therapeutics. However, the amphiphilic nature of these proteins often makes it difficult to investigate their native structures by using conventional structural biology tools.

A synthetic biology approach, in which natural biological systems are redesigned and/or reconstituted, could be an alternative way to unravel the mechanisms of membrane-related biological events. Structural DNA nanotechnology,[1] such as DNA origami,[2] can be a method of choice to produce model structures or mimics of biological macromolecules because such systems are biocompatible, their surfaces and ends can be easily modified, and they can adopt different shapes. A remarkable example is a membrane-spanning channel made from DNA.[3−7] The structure, reported by Simmel and co-workers, was constructed using the scaffolded origami technique and anchored to a lipid membrane by cholesterol-modified strands (Figure 1a).[3] Another strategy developed by Howorka and co-workers was more similar to natural membrane proteins, which have an outer hydrophobic surface.[4−7] In their design, a part of the surface of the six hexagonally arranged interconnected duplexes was modified with a hydrophobic belt consisting of charge-neutral ethyl phosphorothioate[4] or porphyrin groups (Figure 1b).[5] The insertion of these channels and nanopores into lipid membranes was established by electrical measurements.[3−5,7] Furthermore, it has recently been shown that the amphiphilic DNA nanopores exert a cytotoxic effect against HeLa cells.[6]

The successful construction of such membrane-spanning structures has led researchers to undertake further challenges including the creation of systems that imitate other membrane-related biological events or structures by means of DNA nanotechnology.[8−10] One of these approaches involves polymerization of DNA origami nanostructures on a lipid bilayer surface.[8,10] Considering that dynamic transformation of lipid membranes such as vesicle fusion/budding is mediated

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Published online April 16, 2015
10.1021/acsnano.5b01723

by the assembly of membrane-associated proteins, this research direction is important for strategies aimed at regulating membrane dynamics by artificial DNA nanostructures.

In this issue of *ACS Nano*, Kocabey *et al.* report on membrane-assisted assembly of DNA origami nanostructures that can mimic the assembly of membrane-associated proteins.[8] In their study, a rectangular three-layered DNA origami block with dimensions of $60 \times 35 \times 8$ nm^3 was bound to cholesterol-modified oligonucleotides that had been pre-incorporated into a supported lipid membrane. The surface-diffusive origami blocks were then assembled into different superstructures in a programmed manner (end-to-end polymerization or corner-to-corner connection) by the addition of different sets of connector staples (Figure 2a). In addition, the authors aimed to mimic the lattice pattern produced by assembly of triskelion-shaped clathrin molecules. Clathrin is a protein that plays a major role in endocytosis, forming coats around vesicles by polymerizing into a polyhedral lattice consisting of six- and five-sided rings.[11,12] To mimic the three-armed shape of clathrin, a bent DNA origami with dimensions of $70 \times 20 \times 15$ nm^3 was designed. The bent DNA structure was approximately Y-shaped, with one of the arms truncated. A key point of this design is that the remaining arms form a 60° angle with the vertical line. Thus, three Y-shaped DNA origami structures can be associated into homotrimers of which three arms form a 120° angle with respect to each other. The preassembled trimers were anchored to the lipid bilayer by the same strategy used for the origami blocks and then further assembled by the addition of connector oligonucleotides. The successful assembly of membrane-supported arrays consisting of pentagons and hexagons was confirmed by atomic force microscopy imaging.

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A notable finding in this study is that the formation of the origami assembly on small unilamellar vesicles (SUVs) could induce deformation of the SUVs. A transmission electron microscopy image of a SUV having origami block monomers revealed its spherical shape. However, subsequent addition of the connector staples resulted in deformation of the shape or disruption of the SUVs together with the formation of origami arrays on the vesicle surfaces. Although further quantitative experiments are required to elucidate the mechanisms and processes involved in the deformation, the results pave the way for the construction of lipid-membrane-interacting

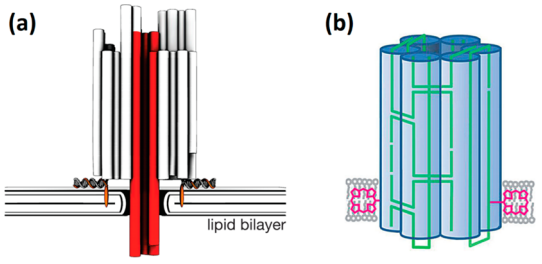

Figure 1. DNA channels penetrating the lipid bilayer. (a) A DNA origami channel anchored to the lipid bilayer using cholesterols. Reproduced with permission from ref 3. Copyright 2012 American Academy for the Advancement of Science. (b) DNA tube channel anchored to the lipid bilayer using porphyrins. Reproduced with permission from ref 5. Copyright 2013 Wiley-VCH Verlag GmbH & Co.

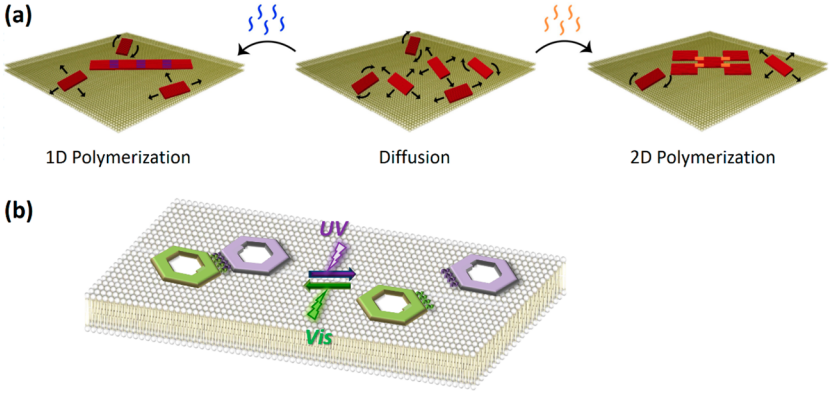

Figure 2. Controlled assembly of DNA origami nanostructures on the lipid bilayer. (a) DNA origami blocks were polymerized into one-dimensional (1D) and two-dimensional (2D) assemblies by addition of specific DNA strands. Reproduced from ref 8. Copyright 2015 American Chemical Society. (b) Hexagonal origami with a photoresponsive system was assembled and disassembled reversibly upon visible and UV light irradiation, respectively.

DNA nanostructures that mimic both the function and the structure of membrane-associated proteins that play a key role in membrane shaping and remodeling.

OUTLOOK AND FUTURE CHALLENGES

The capacity of the origami assembly to deform lipid vesicles reported by Kocabey *et al.* is an important step toward employing DNA nanostructures as components in artificial cells. At this stage, the assembly reaction is not reversible; however, the reversibility can be easily implemented by employing strand-exchange reactions or various switchable DNA systems triggered by metal ions, pH changes, or photonic stimuli.[13−15] In fact, the reversible assembly and disassembly of a cholesterol-modified DNA origami dimer was recently achieved on a lipid bilayer surface[16] by using photoresponsive deoxyoligonucleotides (Figure 2b).[15,17] Speeding up the assembly reaction is also an issue that needs to be addressed. The current bilayer-anchored origami assemblies were obtained after incubation with connector staples for at least 12 h. This is considerably longer than the time required, for example, for the entire process of clathrin-mediated endocytosis (which is on the order of a few tens to hundreds of seconds).[18] In addition to the use of switchable DNA systems, polymers that can accelerate DNA strand-exchange reactions[19] would enable the rate of assembly/disassembly of DNA origami structures to be tuned.

Dynamic changes to the structure of cellular membranes are generally achieved through the interplay between lipids and proteins.[20] It should be noted here that the chemical properties of different acyl chains or head groups of lipids produce different physicochemical properties of membranes. Thus, studies on the lipid-composition-dependent behavior of DNA origami structures and their effects are as important as further functionalization of lipid-interacting origami structures. In the pioneering work performed by Schwille and coworkers, switchable partitioning of DNA origami rods between liquid-ordered/liquid-disordered phases was observed in a magnesium-ion-dependent manner.[21] Elucidation of the molecular origin of such behavior will also be key to the future construction of DNA-based artificial cellular components.

The highly programmable features of DNA molecules could enable the operation of differently functionalized origami structures in a cascading or cooperative manner in/on lipid vesicles. It is hoped that a range of cellular events could be simulated by DNA nanostructures.

Conflict of Interest: The authors declare no competing financial interest.

Acknowledgment. The Authors acknowledge financial support from Core Research for Evolutional Science and Technology (CREST) of JST and a Grant-in-Aid for Scientific Research on Innovative Areas "Molecular Robotics" (No. 24104002) (M.E.) from MEXT, Japan.

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