NANOTECHNOLOGY

Changing of the guard

DNA nanostructures
mimic membrane proteins
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Membrane proteins control access of ions and molecules to a cell's interior , shuttle cargo and information across the cell boundary, and determine the cell's shape. Engineering the function of these proteins is key to the development of vaccines, biofuels, biosensor elements, and research tools. However, the range of accessible architectures is limited, because classical protein engineering usually involves making relatively modest structural changes to existing protein structures; folding extensively altered polypeptides into defined structures is very challenging. Recent studies have shown that some membrane protein functions can be mimicked with nanostructures built from DNA. Such DNA nanostructures are easier to manipulate than their natural templates and can advance research, biotechnology, and synthetic biology.

The programmable base-pairing of DNA has previously been exploited to create nanoscale architectures with angstrom-scale precision (1). These non-membrane DNA origami nanostructures self-assemble from mixtures of component strands, forming interlinked duplexes that act as the main structural domains. Scientists have used this approach to create molecular motors and antibodies.

However, creating biomimetic membrane structures poses an additional challenge: how to insert the hydrophilic, negatively charged DNA into hydrophobic centers of lipid bilayers. This biophysical challenge can be overcome by attaching hydrophobic membrane anchors, such as cholesterol, to the DNA nanostructures, as pio-neered for DNA duplexes (2, 3). The resulting biomimetic architectures float on bilayers without puncturing them (see the figure). These structures are analogous to natural membrane proteins that are components of the cytoskeleton or of signaling cascades.

Self-assembly with DNA is highly modular, allowing membrane-floating nanostructures of different shapes to be built, includ-ing bricks (4), stars (5), and flat rectangles (5, 6). At diameters of up to 80 nm, the structures are larger than amino acid–based engineered membrane proteins and can thus control the shape of lipid vesicles (4, 5). The nanorectangles can be tuned to oligomerize into larger superstructures and undergo a nanoscale shape change (4-6). Furthermore, DNA origami can form ring-like scaffolds as large as 200 nm that enclose vesicles like a ring around Saturn (7, 8). Similarly, spherical DNA

scaffolds can be generated to function as endoskeletons inside vesicles (9). In these cases, tethered lipid anchors (7, 9) or membrane proteins (8) mediate contact between the DNA scaffold and the curved bilayer.

DNA structures can also form mem-brane-puncturing pores (see the figure). These pores mimic natural channels that control transport of water-soluble cargo across biological bilayers. DNA versions have a structural core of several interlinked DNA duplexes that encloses a hollow lumen. Membrane insertion is made possible by modifying the outside of the nanostructure with cholesterol (10) or by chemically altering the DNA backbone to remove negative charges (11). The pore diameter can be tuned by altering the number of DNA duplexes (12). In addition, we have shown that a pore with a six-duplex core can be turned into a synthetic ligand-gated channel by adding a DNA lid at one end; the channel entrance can be reopened with the appropriate ligand (13). This channel is highly selective for the transport of small, charged organic molecules. Engineered DNA pores can also be opened and closed via voltage changes, similar to biological ion channels (14).

These nanostructures are remarkable; DNA would not naturally interact in such a defined manner with bilayers. But there are also important applications. In research, membrane-anchored DNA plates are used as tools to reorganize the fluidity or local morphology of the lipid bilayer (4-9) and thereby influence biological behavior. DNA plates may also be used as molecular peg-boards to present cell-activating proteins in stoichiometrically defined low numbers and at precise geometry and nanoscale distance to each other , which is of importance, for example, in activating immune cells. Furthermore, vesicle-enclosing DNA rings can help induce contact with other vesicles or planar membranes and thereby be an important tool to study membrane fusion (7, 8). In biotechnology, DNA scaffolds can control the size and stabilize bilayer vesicles that serve as bioimaging agents or deliver encapsulated drugs (7, 9). Furthermore, DNA pores could serve as components in portable, label-free biosensors. Currently, protein pores 1 to 2 nm in diameter are used for electrical DNA sequencing, but larger pores are necessary to detect diagnostically important proteins, and DNA nanotechnology is a good route to construct these pores. In addition, ligand-gated DNA channels could be used in vesicles for con-trolled drug release (13).

Membrane DNA nanostructures also expand the toolkit of synthetic biology. In classical synthetic biology, engineered DNA or protein parts are used in their traditional biological roles to create artificial viruses, biocatalytic microcompartments, or cells with designed genetic circuits. The DNA rafts and pores break with this convention by using DNA outside its usual functional spectrum. They thus complement other engineered DNA units that mimic the biological function of soluble proteins in catalysis or signal processing within lipid membrane compartments (15). Currently, these systems are rudimentary but can help us to understand natural ones by providing a simplified and modular model. Variants composed solely of DNA might also shed light on the origin of life.

Even more radical approaches do away with biogenic building blocks and instead incorporate completely synthetic nanomaterials within cellular structures. This is advantageous when the properties of the synthetic component add new functionality. Examples are carbon nanotubes or silicon nanoneedles, which puncture the cellular bilayer to form an electrical interface between the cell's interior and semiconductor chips or to inject nucleic acids for vaccination. Alternatively, membranes can be replaced altogether with polymers to produce synthetic vesicles of greater stability. It is, however, more difficult to achieve biological compatibility with such completely synthetic systems than with DNA nanomaterials.

Future research in this young field will explore DNA designs of different size, geometry, and lipid anchoring. This will help to answer questions concerning how nanostructures interact with membranes and how they deform the bilayer structure. Of additional interest is how the structurally flexible and porous DNA nanostructures compare to more rigid proteins, and how to combine DNA with other protein or polymeric components for hybrid or biomimetic nanostructures It will be a challenge to produce DNA nanomaterials more cheaply in order to realize their potential in biotechnology (1). Finally, for creating self-replicating synthetic cells, membrane-anchored DNA nanostructures will have to be made solely from biological components. This may be achieved by using the amphiphilic nature of DNA. With further progress in these research areas, DNA-based gatekeepers at biological membranes are well positioned to further exploit powerful engineering with DNA nanotechnology.

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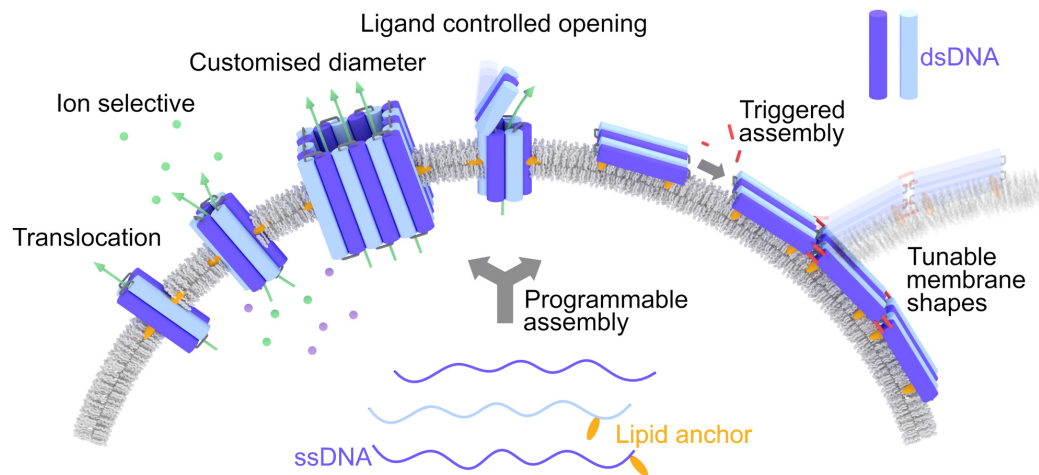
DNA-based gatekeepers. DNA nanostructures have been rationally designed to
form pores and channels or to adhere to the membrane bilayer, thereby
mimicking membrane proteins in living systems. The DNA nanostructures are of
interest for a range of research and biotechnology applications.

Image credit: Jonathan Burns and Adrian Hodel