ABSTRACT: Artificial molecular machines have found widespread applications ranging from fundamental studies to biomedicine. More recent advances in exploiting unique physical and chemical properties of DNA have led to the development of DNA-based artificial molecular machines. The unprecedented programmability of DNA provides a powerful means to design complex and sophisticated DNA-based molecular machines that can exert mechanical force or motion to realize complex tasks in a controllable, modular fashion. This Perspective highlights the potential and strategies to construct artificial molecular machines using double-stranded DNA, functional nucleic acids, and DNA frameworks, which enable improved control over reaction pathways and motion behaviors. We also outline the challenges and opportunities of using DNA-based molecular machines for biophysics, biosensing, and biocomputing.

KEYWORDS: Artificial molecular machines, DNA nanotechnology, DNA framework, Functional nucleic acids, Nanomedicine

1. INTRODUCTION

Natural cellular machinery is essential for the function and processivity of a wide variety of biologic processes, ranging from adenosine triphosphate (ATP) synthase at the nanoscale to cell recognition at the microscale. In general, machinery is composed of a multitude of functional components working in tandem. These primitive biomolecules, in particular nucleic acids and proteins, exert mechanical force or motion in response to certain external stimuli (signal input). Inspired by these natural biomolecular machines, many researchers have repurposed artificial biomolecules to engineer artificial molecular machines that execute a list of functions and accomplish complex tasks in a controllable, modular manner. These studies lead to not only advancements in the creation of synthetic cells and lifelike entities but also insights into the structure and functionality of artificial molecular machines that have broad implications for a variety of fields ranging from fundamental biology to biomedical applications, including biomedical engineering, biosensing, and theragnostics.

Since the 1980s, a variety of chemicals and materials, including small organic molecules, nanoparticles, proteins, RNA, and DNA, have been utilized to construct diverse artificial molecular machines. As an exquisite illustration, Feringa and colleagues created organic molecule-based molecular machinery with a unidirectional rotation that performs complex tasks utilizing chemical fuels, light, or electrochemical react ions, as recognized by the 2016 Nobel Prize in Chemistry. Alternatively, DNA has also widened our vision and facilitated the development of artificial molecular machines. In particular, since Seeman’s pioneering work in the early 1980s, rapidly growing structural DNA nanotechnology has opened up new possibilities for the building of static and dynamic 2D and 3D DNA-based molecular machines (DMMs), that are built from exquisite architectures of DNA sequences and achieve mechanical operation by DNA in a dynamic, controllable, modular manner. Due to the flexible backbone of ssDNA and the rigid framework of double-helical DNA domains, the local stiffnesses of DNA nanostructures can be programmed and coupled to fit a wide range of structural requirements for each particular molecular machine design. Meanwhile, by exploring DNA’s highly predictable assembly qualities and the programmability of its hybridization processes, DNA offers a path for programming and controlling complicated motion or behavior at the nanoscale. In addition, the diverse kinds of DNA secondary structures found in nature, such as G-quadruplexes and i-motifs, offer an exquisite approach to controlling the functionalities and behaviors of DMMs. Thus, DNA rapidly evolved into this booming field of artificial molecular machine construction, closely resembling natural protein motors in terms of nanoscale size.
With the development of DNA nanotechnology, initial research focused on creating and fabricating DMMs. To develop advanced applications, these DMMs must operate at the interface rather than in the liquid. The supporting interface, which is typically where biological systems or cascade-triggering events are integrated, would enable evaluating its functionality, such as direction control and programmability. Specifically, because unique DNA can be programmed in a variety of ways, localized motors on the surface are far more likely to be triggered by adjacent molecules or output signals, allowing for better control over reaction pathway direction. Moreover, once they are anchored to solid or soft surfaces, anchoring, addressability, and cooperative operation become critical, which provides a path to system complexity and considerable promise for the realization of functional DMMs.

In this Perspective, we focus on recent advances in DMMs. We first introduce the progress of DNA nanotechnology in the construction of DMMs and then describe methods for integrating DMMs at the interface as well as tactics for regulating motion and behavior. Following that, we provide a detailed review of recent reports on its biological application in biological rulers, biologic factories, biocomputing, and biosensing. Last, we discussed the future challenges and perspectives of this field.

2. DNA NANOTECHNOLOGY-ENABLED DMMs

2.1. DNA Nanotechnology

DNA is a lengthy natural biopolymer made up of four distinct bases: adenine (A), thymine (T), guanine (G), and cytosine (C), which constitute the basis for genetic information in living organisms. According to the Watson–Crick rule, the DNA strand hybridizes to its complementary sequences to form the B-form double helix structure, and the formed double helix structure has a diameter of 2 nm and a helix turn of 3.4 nm for every 10.5 base pairs. Seeman established in the 1980s that double-stranded DNA is also an ideal nanoscale building block via extremely sequence-specific hybridization. He suggested that arbitrary-shaped DNA nanostructures may be self-assembled by programming an immobile Holliday junction branching motif, and he succeeded in creating 2D and 3D DNA lattices beyond 1D linear double-strand DNA. Since then, advancements in structural DNA nanotechnology have enabled the fabrication of a range of intricate 2D networks and 3D DNA nanostructures, such as tetrahedrons and cubes, ushering in a new era of structural DNA nanotechnology (Figure 1).

Figure 1. Timeline of tile-based and origami-based DNA nanostructures: (1) 2D DNA tiles including one-arm junction. Reproduced from ref 199. Copyright 2019, American Chemical Society. (2) DNA cube. Reproduced from ref 199. Copyright 2019, American Chemical Society. (3) Double-crossover (DX)-based alternating 2D array. Reproduced from ref 200. Copyright 2021, American Chemical Society. (4) 2D square lattice assembled with 4 × 4 DNA tiles. Reproduced from ref 199. Copyright 2019, American Chemical Society. (5) Tetrahedral DNA framework. Reproduced from ref 199. Copyright 2019, American Chemical Society. (6) Hierarchical polyhedral DNA framework. Reproduced from ref 199. Copyright 2019, American Chemical Society. (7) 3D DNA crystal self-assembled with a tensegrity DNA triangle motif. Reproduced from ref 199. Copyright 2019, American Chemical Society. (8) Tile DNA bricks-based 3D DNA framework. Reproduced from ref 199. Copyright 1999, American Chemical Society. (9) 3D DNA teddy bear. Reproduced from ref 201. Copyright 2021, American Chemical Society. (10) DNA-origami-based smiley face. Reproduced from ref 199. Copyright 1999, American Chemical Society. (11) 3D Hollow DNA box with a lid. Reproduced from ref 200. Copyright 2021, American Chemical Society. (12) 3D monolith with multiple pleated layers. Reproduced from ref 199. Copyright 2019, American Chemical Society. (13) 3D ellipsoid with complex curvature. Reproduced from ref 199. Copyright 2019, American Chemical Society. (14) Fractal assembly of DNA origami arrays with arbitrary patterns. Reproduced from ref 200. Copyright 2021, American Chemical Society. (15) Gigadalton-scale 3D dodecahedron. Reproduced from ref 200. Copyright 2021, American Chemical Society. (16) Double stranded meta-DNA. Reproduced with permission from ref 61. Copyright 2020, Nature Publishing Group.
enables the creation of different tile-based DNA nanostructures, such as DNA cube, single-step formation of DNA tetrahedrons, hierarchical polyhedral DNA structures, and DNA branched junctions with 5-, 6-, 8-, and 12-arm double-helical arms surrounding a branch point.\textsuperscript{45–48} Following this remarkable development, a variety of DNA tiles were self-assembled into periodic 2D and 3D lattices with a proper sticky-ends design.\textsuperscript{40} Moreover, because the sizes of periodic 2D and 3D crystals are potentially infinite, rationally designed crystals with sizes of hundreds of micrometers were formed using man-made DNA tiles in 2009.\textsuperscript{40–44} Specifically, Seeman and co-workers created the first diffracting DNA crystal by employing a tensegrity rigid triangle motif with 3-fold rotational symmetry.

Furthermore, Yin et al. further introduced a chimeric conceptual form of DNA tile in 2008, dubbed double-stranded tile (SST), which is a 42-base-long single-stranded DNA.\textsuperscript{49} Rather than containing at least two or more DNA strands, the SST was composed of four concatenated sequences of nearly identical length. They were shown to assemble long tube circumferences by binding to their four local neighbors SST via their four concatenated motif. In 2012, they further demonstrated a conceptual extension of the SST, dubbed the ‘DNA brick’, which is a 32-nt-long single-stranded DNA.\textsuperscript{50,51}

By connecting to the four nearest neighbors in a square lattice arrangement of parallel helices, these DNA bricks were shown to form a variety of DNA nanostructures with 2D and 3D arbitrary shapes as well as crystal lattices with channels and pores.

In 2004, Paul Rothemund introduced a different approach, dubbed “scaffolded DNA origami”, which represents another major advance in structural DNA nanotechnology.\textsuperscript{52} In scaffolded DNA origami, a long single-strand DNA (ssDNA) oligomer was employed as a scaffold on which many DNA staple strands were stapled into desired forms in a one-pot method. This approach enables the construction of static DNA frameworks with predetermined sizes ranging from 50 to 500 nm with unprecedented precision and yield.\textsuperscript{53–57} Moreover, the local stiffness of a DNA origami nanostructure can be fine-tuned by programming its persistence length, allowing for the building of rigid and static designs. Furthermore, because these DNA staple strands are chemically generated with precise sequences, they can be changed and coupled to a variety of functional molecules, including enzymes, nanoparticles, and tiny compounds. Thus, the use of DNA origami nanostructures realizes the nanoscale control of molecular positioning and dynamic motion.

High-order hierarchical and large-sized DNA-framework constructions have become achievable by the stepwise assembly of DNA frameworks.\textsuperscript{56,59,63,81} One major approach to scaling up the DNA framework is the sticky end hybridization method, in which each DNA origami edge is extended with complementary ssDNA for more on-demand hybridization. Qian et al. used this process to generate larger 2D DNA origami 10 × 10 arrays with numerous square origami tiles, reaching 880 × 880 nm\textsuperscript{2}. Following that, she then developed a “fractal assembly” with a three-stage self-assembly approach to produce a sophisticated DNA origami array with a maximum resolution of 8704 pixels and a size of 3.5 × 4.5 μm\textsuperscript{2}. In addition, blunt end stacking was a sophisticated alternative method for scaling up DNA nanostructures. Similar to the key-and-lock concept, blunt end stacking is based on several DNA nanostructures docking in complementary shapes. As proven by Dietz et al., a microscale 3D rhombohedral nanostructure up to 2.5 μm in size has been successfully built.\textsuperscript{60} Furthermore, Our group introduced M-DNA, a six-helix DNA origami bundle as a basic building block, to assemble a scaling-up DNA framework with sizes up to sub-micrometers and micrometers.\textsuperscript{61} Research on the scaling up of DNA frameworks has enabled the development of resilient hierarchical DNA architectures.

### 2.2. DMM Construction

The booming advancements in structural DNA nanotechnology have attracted worldwide interest in harnessing DNA’s extraordinary architectural precision. Nature harnesses the modular framework to integrate numerous functions into natural molecular machines and perform complex tasks in tandem. This principle inspired the utilization of framework nucleic acids (FNA), including double-stranded DNA and static DNA frameworks, to build integrated and functional DMMs.\textsuperscript{62,63} By exploiting the programmability of structural DNA frameworks, the FNA enables the precise spatial arrangement of oligonucleotides in the modular DNA framework at the nanoscale. Moreover, owing to the advancements in the chemical synthesis of DNA, a variety of chemical groups, including fluorescent dyes, chemical-stimulus responsive chemicals, proteins, and nanoparticles, can be site-specifically modified at the ends of oligonucleotides.\textsuperscript{64–66}

Furthermore, the intrinsic biocompatibility and biodegradability of FNA have made it a perfect biomaterial for programming DMM’s logical response to various chemical stimuli.\textsuperscript{67} Thus, the FNA features as an ideal molecular template for spatially organizing various functional molecules and materials in vitro and in vivo, presenting a promising strategy for developing DMM with integrated functions.

An interesting extension of this research is to transform FNA into a molecular machine.\textsuperscript{68,69} In 1999, Seeman and colleagues leveraged the sequence specificity of DNA and demonstrated the concept of DMMs.\textsuperscript{70} They developed dynamic DMMs capable of performing similar motions, as they demonstrated using the B-Z transition of DNA to switch the conformations of two stiff DNA “double-crossover” molecules. Hence, the incorporation of stimuli-responsive components into static DNA nanostructures may enable the generation of a range of sophisticated nanomechanical motions. Hereby, we focused on introducing several reconfigurable elements that have been incorporated into the DNA frameworks (Figure 2).

Much of the effort in engineering dynamic DMMs focused on integrating the Toehold mediated DNA strand displacement (TMSD) element into static DNA nanostructures, which was established by Yurke et al. in 2000.\textsuperscript{71} The TMSD typically begins with an ssDNA coupled to a partly dsDNA in its unhybridized domain. As a result of this process, the ssDNA undergoes branch migration to completely exchange the complementary ssDNA from the dsDNA.\textsuperscript{72} They used this technology to design a pair of DNA tweezers composed of two DNA helices and used an auxiliary strand of DNA to open and close them, resulting in the successful fabrication of a reversible DNA motor (Figure 3a).\textsuperscript{71} Recently, the TMSD approach has been used to reconfigure DNA origami nanostructures and lead to the creation of various complex and hieratical DMMs, such as nanoboxes, nanotweezers, and nanotubes. Moreover, by utilizing ssDNA as a fuel source, this strategy opened up new means for the building of processive DMMs with real-time coordinated motion. These accomplishments pave the way for
Toehold mediated DNA strand displacement. (a) Schematic images of the reversible DNA tweezers by TMSD. Reproduced with permission from ref 71. Copyright 2000, Nature Publishing Group. (b) Schematic and AFM images of DNA origami Möbius strip. Reproduced with permission from ref 73. Copyright 2010, Nature Publishing Group.

Figure 3. Toehold mediated DNA strand displacement (TMSD). (a) Schematic images of the reversible DNA tweezers by TMSD. Reproduced with permission from ref 71. Copyright 2000, Nature Publishing Group. (b) Schematic and AFM images of DNA origami Möbius strip. Reproduced with permission from ref 73. Copyright 2010, Nature Publishing Group.

Figure 2. Reconfigurable elements and strategies for building DMMs. (a) Toehold mediated DNA strand displacement. (b) Degradation of DNA strands. (c) Special DNA motifs or target binding aptamers that undergo a conformational switch in response to environmental cues.

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The development of a library of programmable DMMs with modular and kinematic joints akin to those seen in macroscopic machines. Han et al., for example, used the TMSD approach to induce structural change in the DNA origami Möbius strip, a topological ribbon-like structure with a single side (Figure 3b). 73 Using the TMSD approach, the DNA Möbius strip can generate about the double-sized topological supercoiled ring and catenane structures. Owing to its robustness in a wide range of temperatures and multiplexed nanosystems, the TMSD approach has been one of the most commonly used and established methods for controlling the motion of dynamic DNA nanostructures and DMMs. 74

Furthermore, enzymatic reactions, such as cleavage, degradation, and extension, are another versatile approach for manipulating reconfigurable components in DMMs. Rather than relying on strand displacement, this approach uses a variety of enzymes, including nucleases, DNAzymes, and polymerases, to trigger the release or capture of DNA oligomers, thereby driving DMMs to perform mechanic motion tasks. 75 Because an enzymatic process is deemed autonomous and irreversible until no substrate is available, this technique is most frequently used to build a DNA walker, which typically comprises three essential complements: driving force, walking foot, and DNA track. Reif et al. reported the first enzymatic reaction-mediated DNA walker. 76 Its walking strand, in particular, contains a short DNA fragment that connects to two distinct types of footholds that are attached at regular intervals along the DNA track. When the walking strand hybridizes with the foothold, a nick is formed, exposing the enzyme and initiating DNA cleavage. To ensure the DNA walker moved in a directed fashion, they employed two nicking enzymes, PflM I and BstAP I, and destroy the restriction sites of the DNA. Following this cycle, the walker proceeds along with the foothold until he reaches the track’s conclusion.

The concept of enzymatic reaction induced strand migration for DNA walkers was further developed by incorporating the DNAzyme system, which catalyzes the cleavage of a ribonucleotide phosphodiester link in ssDNA via a trans-esterification reaction. 77 Due to DNAzyme’s superior performance with quicker strand displacement rates, the DNA walker displayed enhanced mobility but decreased attachment stability to shorter legs, driving the DNA walker moving along the DNA track. In 2005, Mao et al. demonstrated that the walking strand can be driven by DNAzyme cleavage of a specific RNA sequence (Figure 4a). 78 Moreover, the motility and processivity of DNAzyme-based DNA walkers can be programmed via various parameters, including the length of the substrate-binding legs, the track type, and the DNAzyme structure. Willner et al. recently demonstrated the incorporation of a mixture of origami tiles into Zn2+- and/or Pb2+-dependent DNAzymes in order to program the operation of transformations in the origami nanocavities’ nanoholes (Figure 4b). 79 Since DNAzyme is amenable to combinatorial selection, the incorporation of DNAzyme into the DNA motor not only broadens the range of work conditions available to the protein enzyme but also introduces a novel technique for DMMs with definable landscapes and programmable behavior.

In addition, functional nucleic acids, such as DNA triplex, aptamer, G-quadruplex, or i-motif, can be used as a reversible functional module or actuator in the construction of dynamic and sophisticated DMMs. 80−83 The conformational transition of functional nucleic acids generates the mechanical force, enabling to drive the internal movement of the DMMs’ individual static nanostructures. Moreover, the incorporation of functional nucleic acids into dynamic DMMs enables unique controllability in response to a variety of ambient chemical stimuli, including pH, ATP, light, and Na+, as well as external physical cues, including light, thermal, electric, and magnetic fields. For example, Kuzuya et al. developed a DNA origami
DNA origami plier with shape transitions between closed and open states (Figure 5a). By incorporating different elements that bind together in the presence of the target into each of the levers, the DNA origami plier can be used as single-molecule beacons to visually detect a variety of biomolecules, including streptavidin, IgG, Ag⁺, and miRNA, via atomic force microscopy (AFM). Using this similar mechanism, we also designed a DNA origami nanocaliper as a dynamic shape-resolved nanodevice for pH sensing at the nanoscale (Figure 5b). By integrating pH-responsive Triplex DNA into the two arms of the origami nanocaliper, it can change its shape in response to changes in the local pH and act as a readout for transmission electron microscopy (TEM) imaging.

Furthermore, the light-induced conformational switch can also be employed to control the actuation of DMM. One major approach was to modify the DNA backbone with ortho-nitrobenzyl or azobenzene groups, which undergo isomerization when exposed to UV light. Particularly, the melting temperature of modified dsDNA was dominated by the photocleavage of ortho-nitrobenzyl or conformational switch of azobenzene-DNA, providing a means to control its association and dissociation with exposure to UV. For example, Han et al. demonstrated a structural reconfiguration of DNA origami sphere by modifying photocleavable ortho-nitrobenzyl between adjacent DNA helix (Figure 5c). The DNA origami sphere split into two hemispheres when exposed to light. Although light-based actuation is inefficient and slow, it has significant potential in biomedicine due to its noninvasive and conventional nature.

3. DMMs AT THE INTERFACE

The incorporation of DMMs at the interface, rather than in the liquid, allows for the harnessing of nucleic acid structure and functioning. This approach further enables DMM to engineer the addressability and cooperative operation at the interface, enabling DMMs to achieve sophisticated tasks in a controllable, modular manner. The developments in solid-phase chemical synthesis of ssDNA have provided a convenient means for the synthesis of DNA with customized functionality. Specifically, diverse functional groups or domains can be modified at multiple sites of ssDNA, particularly the 5′-terminal and 3′-terminal of the ribose backbone. This customized DNA further enables the design of DMMs with unique functionality and additional properties, such as label attachment and surface anchoring. With these achievements in DNA modification and structural DNA technology, DMMs have been incorporated onto different solid and soft surfaces, which is a promising step toward realizing DMMs with controllable function (Figure 6).

3.1. Nanoparticle

The physicochemical features of nanoparticles include size-dependent optical and electrical properties. The anchoring of DMMs onto the surface of nanoparticles results in the creation of innovative hybrid systems, which allow to incorporate the advantageous properties of nanoparticles and DMMs. This endows DMMs with novel capabilities and functionalities, such as addressability and in vivo stability.

One of the most impressive demonstrations of these DMMs on the gold nanoparticle (AuNP) surface is the AuNP-based core–satellite like nanosystem by Chan et al. (Figure 7). This nanosystem consists of a core nanoparticle surrounded by small satellites, and its conformation can be transformed in response to DNA via a toehold displacement mechanism, resulting alteration of its optical properties and biological interactions of the assembled nanosystem to the cell. Specifically, the DNA monolayer formed on the AuNP’s surface regulates the configuration of AuNP assemblies, enabling the assembly of shape-shifting DMMs with controlled biological functions.

3.2. DNA Framework

For DMMs, nanoscale function control is confined to stabilizing various preprogrammed states, rather than operating the device directly and continuously with applied force, such as

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Figure 5. Functional nucleic acids actuation by chemical or physical cues. (a) Shape transitions of nanosized pinching DNA origami devices by a variety of chemical cues, such as ATP, Na⁺, Ag⁺, and miRNA. Reproduced with permission from ref 84. Copyright 2011, Nature Publishing Group. (b) DNA origami nanocaliper actuated through the incorporation of the pH-responsive i-motif sequence. Reproduced with permission from ref 85. Copyright 2022, Royal Society of Chemistry. (c) Schematic and TEM image of the light reconfigurable DNA origami sphere. Reproduced with permission from ref 87. Copyright 2015, Royal Society of Chemistry.

Figure 6. Substrates for DMM surface attachment. Harnessing the unique functionality and additional properties of DMM by immobilizing them at various interfaces.

Figure 7. Perspective
The American Association for the Advancement of Science.

by DMM. Reproduced with permission from ref 100. Copyright 2016,

time manipulation of DMMs with high spatial precision,
electronic and magnetic fields or directional flow. Direct real-
time manipulation of DMMs with high spatial precision,
subsecond response times, and customizable applied forces is a
critical task for creating them. Apart from being used to build
artificial molecular machines, DNA frameworks can also be
employed to build tightly coupled DMMs. By precisely
programming the DNA origami landscape and operation
units of molecular machines, the incorporation of DMMs at
the interface of DNA frameworks provides a promising means
to programming its motion, in which the position or
orientation of DMMs changes over time. The control of the
motions enables a variety of functions, such as spatial
manipulation of functional molecules, logic circuits, parallel
processing, and signal transduction. Thus, the utilization of
FNA permits the creation of highly ordered and tightly
programmable surfaces with directional motion, and excep-
tional structural and functional programmability.

For the DMMs, translational motion and rotational motion
represent the fundamental directional motion which lays the
foundation for their different complex motions and behav-
iors. Translation, in which an object moves in a given
direction, is the first motion achieved in the research of DMM.
One of its remarkable examples is represented by the DNA
walker, which was demonstrated by Reif et al. and Pierce and
Shin in 2004.\textsuperscript{76,103} The DNA walker mostly consists of two
components: the DNA walking strand and the DNA track.
Typically, the walking strand continuously undergoes a
nanoscale change of the position along the DNA track, similar
to the DNA polymerase along with the DNA template. The
continuous locomotion of the DNA walker is realized by
transforming a variety of energy to disrupt the initial
equilibrium of DNA interaction and progress down the DNA
track. This walking mechanism endows DNA walkers with the
fundamental properties of molecular machines, such as
progressivity, processivity, and directionality.\textsuperscript{104,105} Since the
pioneering work of Reif et al., most researchers designed
bipedal walkers that used toehold-mediated strand displace-
ment to remove the linker between the walker foot and track.
To actuate the molecular machine, the second set of DNA
linkers was added to the system and its hybridization resulted
in the detachment of the foot and pairing to the next available
foothold.

With the development of structural DNA nanotechnology,
DNA origami offers opportunities to design and preprogram
DNA walkers on the customized track with arbitrary shapes
and structures. By exploiting the addressability of DNA
nanostructures, different DNA walkers with autonomous
motion over linear, 2D and 3D DNA ‘tracks’ have been
achieved. As a remarkable example, Yan et al. programmed the
directional movement of DNA walkers by precisely defining
the track on the DNA origami (Figure 8a).\textsuperscript{106} To ensure the

walking strand anchoring on the surface track and moving
route in the desired route, the walking strand was further
designed with three DNA feet. Since each DNA foot moved
independently along the DNA track, the cooperation of these
three DNA feet would avoid the walking strand to be released
from the origami DNA interface. Once actuated by the
cleavage of foothold on the DNA track, the programmed
translational motion is achieved by the recycled binding and
cleaving of these legs. Meanwhile, Walter et al. optimized DNA
walker with cartwheeling movements along the track on the
DNA origami surface. This resulted in a significantly enhanced
reaction speed of hybridization reactions, approximately 1 s\textsuperscript{−1}
for a stepping rate constant, which is 10–100 times faster than
that of previous DNA walkers (Figure 8b).\textsuperscript{107} This facilitated
hybridization reaction further ensures the DNA walking strand
moving along the DNA track.

In addition, interlocked-molecule-based DMMs, such as
catenanes and rotaxanes, also have attracted interest, due to
their potential as molecular cargo shuttles to transport cargo
along a predetermined track.\textsuperscript{108,109} For instance, Lin et al.
engineered a DNA-origami-based rotaxane with program-

Figure 7. Harnessing of property of nanoparticles by modifying
DMMs at their surface. Schematic illustration and corresponding
TEM images of the shape change of nanoparticle assemblies mediated
by DMM. Reproduced with permission from ref 100. Copyright 2016,
The American Association for the Advancement of Science.

Figure 8. Translational movement at the DNA origami surface. (a)
The walker directionally moves on a DNA origami track, hydrolyzing
the complementary footholds. Reproduced with permission from ref
106. Copyright 2010, Nature Publishing Group. (b) Exploring the
speed limit of toehold exchange on a DNA origami track. Reproduced
with permission from ref 107. Copyright 2018, Nature Publishing
Group.
mable transitional motion.\textsuperscript{110} Meanwhile, Simmel et al. created a large, rigid rotaxane using DNA origami, and a long-range transitional motion of up to 355 nm was realized.\textsuperscript{111} Therefore, not only do the nanostructure surfaces topologically arrange these supported DMMs in the desired pattern and route, but also they enable nucleotide-level manipulation of their structure and function, allowing for the performance of specific tasks at the nanoscale by these well-controlled DMMs.

In contrast to translational motion, rotational motion means circular movement around a fixed point. For DMMs, it generally operates by cycling through several multiple preprogrammed states in the response to energetic stimulation or the availability of a suitable fuel. To precisely control the specific configuration of DNA rotary machines, the pivot point for working components should be fixed on the anchoring surface. Dietz et al. constructed a fully synthetic DNA origami with different multilayers consisting of a rotor unit, which is powered by thermal fluctuation. The rotor has a hexagonal cross section and features a crank-lever-like axial protrusion and a body element that forms an axle bearing. The apparatus with greater structural complexity than previous mechanically interlocked objects reproduce some of the dynamic properties of the F1 ATP synthase motor and features a well-defined angular degree of freedom without restricting the range of rotation (Figure 9a).\textsuperscript{112} This system inspired new types of single-molecule assays by installing receptors in the bearing cavity and their ligands on the rotor due to the modularity. Moreover, Murata et al. further designed a thermal fluctuation powered DNA nanostructure with a rotor immobilized onto a substrate surface to evaluate its stepping operation (Figure 9b).\textsuperscript{113} Unlike the rotary motor presented by Dietz, this motor has a dynamic state (in which the rotor rotates randomly) and a static state (in which the rotor is stationary) at predetermined angles. Meanwhile, these states can be selected by a signal DNA strand given from the outside. These intrinsic DMMs demonstrate the importance of the surface to the controllability of the rotational motion.

In addition, the direct real-time manipulation of DNA nanodevices with exact spatial resolution, subsecond response times, and customizable applied pressures is a critical task for mechanical robot engineering. And this is a critical step toward enabling practical robotic systems at the molecular level using DMMs. Inspired by the design of macroscopic motors, starting with isolated joints for angular or linear motion, anchoring the pivot point for working components on the DNA nanostructure’s surface enables fine control of the DNA rotary motor’s particular configuration. Meanwhile, these rotaries can be manipulated using a variety of forces, including chemical forces associated with DNA displacement and electromagnetic forces.\textsuperscript{114,115} Simmel et al. used a computer-controlled electrical field to precisely switch the arm between arbitrary positions on the platform within milliseconds (Figure 10a).\textsuperscript{116}

The DMM is at least 5 orders of magnitude faster than that previously reported for the fastest DNA motor systems and comparable to naturally occurring adenosine triphosphatase-driven biohybrids. Moreover, Castro et al. used magnetic force to control the specific motion of a rotary via a magnetic bead conjugated robotic arm by connecting a stiff microscale mechanical lever to the DNA nanostructure interface (Figure 10b).\textsuperscript{114} Its conformational change as well as the moment generated by the magnetic force can be visualized directly. Furthermore, Rant et al. demonstrated persistent orientation switching in a model DNA rotary that is end-tethered to a metal electrode and actuated by external voltages.\textsuperscript{117} They discovered that applying positive potentials to the surface facilitates selective immobilization of DNA nanostructures, allowing for the attachment of origamis in the presence of an abundance of staple strands from the solution reaction. These studies demonstrate that the DNA nanostructure surface paves the way for the development and characterization of a library of tunable DNA origami kinematic joints and their application in more complex controllable mechanisms resembling macroscopic machines.

3.3. Cell and Lipid Membrane

Micelles, vesicles, and membranes derived from lipids provide a unique surface for anchoring biomolecules, allowing functionally different compartments to be separated from one another. The lipid bilayer is characterized with a dynamic surface. The advantages of the lipid bilayer are its ease of preparation, its ability to build massive molecular assemblies (up to the micrometer scale), and the embedded components’
mobility. Hybrid systems composed of DNA nanostructures and lipids have been designed to exploit the physicochemical properties of membrane assemblies.\(^{62,118}\) Moreover, organizing DMMs within membranes reduces their dimensionality and enables them to interact more efficiently than when they are organized in three dimensions.\(^{119–123}\)

To simulate intracellular communication and transport in cells, DMMs can be attached to lipid bilayer membranes via electrostatic attraction or by functionalization with membrane-interacting molecules, simulating membrane-associated proteins. In general, *de novo* construction of DNA nanostructures up to tens of nanometers in length is comparable to the natural proteins and polypeptides, such as tiny component sizes and short persistence. Existing DNA nanopores are mostly constructed using parallel-aligned DNA duplexes with optional curved sections serving as scaffolds. The scaffolds, in particular, contain lipid anchors such as cholesterol, porphyrin, or tocopherol, either on their membrane-spanning outer pore wall or in places next to the bilayer head groups, allowing for a secure attachment to the lipid surface. A porphyrin-functionalized DNA-based hexagon was one of the first examples of a DNA nanostructure interacting with a lipid bilayer membrane. Simmel et al. used scaffolded DNA origami to produce the artificial membrane channel structure (Figure 11a).\(^{124}\) The bottom of the cap structure was functionalized with cholesterol to bind it to the membrane, allowing it to successfully

Figure 11. DMM-based synthetic molecular device and engineered cells. (a) DNA-based membrane channel. Reproduced with permission from ref 124. Copyright 2012, The American Association for the Advancement of Science. (b) A rationally designed DNA nanopore features a nanomechanical and sequence-specific gate to regulate transmembrane flux. Reproduced with permission from ref 125. Copyright 2016, Nature Publishing Group. (c) Detachment and hierarchical assembly of DMMs at the cell surface. Reproduced with permission from ref 128. Copyright 2017, John Wiley & Sons, Inc. (d) DNA gelation-based cloaking and declocking of CTCs. Reproduced from ref 129. Copyright 2017, American Chemical Society. (e) Anchoring and operation scheme of DNA probe on the live cell membrane. Reproduced with permission from ref 130. Copyright 2017, Nature Publishing Group.
penetrate lipid bilayer membranes during electrical recordings. In addition, Howorka et al. used DNA structure to develop an automatically determined molecular valve capable of regulating when and which cargo is carried across a bilayer (Figure 11b).\textsuperscript{125} The valve can bind a specific ligand and undergo a nanomechanical change in reaction, therefore opening the membrane-spanning channel. These DNA nanopores can differentiate with high selectivity the transport of small organic molecules that differ in their presentation of a positively or negatively charged group due to the narrow valve and DNA characteristic.

Another intriguing application for embedding DMMs on the cell surface is to engineer the function of the cell membrane. For example, Castro et al. used a DNA nanoplatform to build higher-order DNA assemblies on the cell surface and program cell–cell adhesion between homotypic and heterotypic cells via sequence-specific DNA hybridization (Figure 11c).\textsuperscript{128} This incorporation of DNA origami transforms the cell membrane into an engineered material capable of simulating, manipulating, and quantifying biophysical and biochemical functions found within plasma membranes of living cells. The robust membrane functionalization across epithelial, mesenchymal, and nonadherent immune cells further expands the enormous potential in biological engineering. For instance, our group demonstrated an aptamer-triggered leashed hybridization chain reaction (HCR)-based DMMs for in situ cloaking (or decloaking) of circulating tumor cells (CTCs) by forming porous DNA hydrogels (Figure 11d).\textsuperscript{129} DMMs containing aptamer-toehold billocks specifically recognize epithelial cell adhesion molecule (EpCAM) on the CTC surface, initiating subsequent automated DNA polymerization (HCR) via toehold-initiated branch migration. DMMs also report biological events in living cells. For instance, Tan et al. demonstrated a novel DNA walker capable of transducing transient membrane encounter events into readable cumulative fluorescence signals (Figure 11e).\textsuperscript{130} The DNA walkers translocate from one anchor site to another via a toehold-mediated DNA strand displacement reaction. The DMMs revealed the rapid encounter events of membrane lipid domains and determined their rates and preferences during various live cell membrane signaling events.

### 3.4. Flat Surface

One of the primary goals of DMMs is to make them suitable for macroscopic applications. Although functional DMMs and a variety of other molecular machines have been developed recently and could provide a wealth of components for devices, the majority of these investigations have been conducted in solution. The difficult issue remains that when machines are fastened to a flat surface, their behavior may deviate dramatically from the behavior originally specified in the solution. For example, the motor may entirely cease to function. To overcome this issue, DNA nanostructures have been used to control the density of functional probe components fixed on the surface, hence enhancing molecular recognition capability at the surface.\textsuperscript{131}−\textsuperscript{135} One of the most impressive demonstrations of a DNA switch on the macro surface is the DNA tetrahedral-based molecular pump (Figure 12a).\textsuperscript{134} This pump system was composed of a molecular scaffold, that could serve as the scaffold to ensure highly ordered orientation and spatial isolation of this nanomotor on a macroscopic gold surface, and a configurable motif within responsive edge. By incorporating a pH-sensitive i-motif sequence in the responsive edges, the proton-driven DNA tetrahedral machines can reversibly pump water and ferricyanide in response to environmental pH variation in a noncontinuous flow manner. In particular, it is probably even more intriguing to study water pumps of aquaporins, which are naturally existing proteins that channel water in and out of cells in an organized manner. Meanwhile, Liu et al. demonstrated a light-driven plasmonic DNA switch that can amplify the molecular motion of azobenzene through the host nanostructure and consequently translate it into reversible chiptopical function with large amplitude modulation.\textsuperscript{135}

Furthermore, by converting chemical energy into controlled motion, DNA walkers could be of use in applications on the macroscopic surface such as next-generation sensors, drug-delivery platforms, and biological computing. For example, Salaita et al. demonstrated a DNA walker made from DNA coated spherical particles that hybridize to a surface coated with complementary RNA (Figure 12b).\textsuperscript{136} The motion is achieved through the addition of RNase H, which selectively hydrolyzes the hybridized RNA. In particular, the DNA walker rolls rather than walks in a self-avoiding manner, and anisotropic particles can travel linearly without a track or external force. As the rolling mechanism of motion is highly sensitive to external stimuli, such as ATP and miRNA, the DNA walker represents an important step in bringing together the field of DNA-based sensing with the emerging area of DMMs, enabling one to engineer label free sensing assays. The demonstrated strategy exhibited excellent detection performances for DNA analysis in a complex matrix such as human serum, which illuminated the practical application field of the sensing platform.

### 4. APPLICATIONS OF DMMs

Precision positioning of functional molecules is necessary for DMM to integrate and support multiple functions. By acting as a molecular landmark, the positional accuracy of DMM measurements at the nanoscale is a valuable tool for understanding biomolecular interaction at the interface. Moreover, the structural versatility and programmability of...
DNA nanostructures enable nanometer-precision positioning and coordination of the motions of diverse molecules at the interface, enabling the realization of advanced applications such as molecular synthesis, logic circuits, parallel processing, and signal transduction. DMMs could be used as imaging probes and drug carriers in living systems for nanomedicine, as one application. We have demonstrated extensive control over the motion of DMMs at the interface by constructing a biological network for biocomputing.

4.1. Biomolecular Ruler

To perform functions and regulate states, proteins in cells rely on their nanoscale distribution and rearrangement. Due to the precise positioning of multiple biological molecules, DMM has been used as a "molecular breadboard" to answer stoichiometric and quantitative questions about biomolecular interaction over the past decade. The DMM converts its structure or conformation into a biomolecular interaction or dissociation event, which is analyzed using force spectroscopy, by placing and arranging functional biomolecules in a predetermined pattern on addressable 2D and 3D DNA origami scaffolds. Probing protein assembly at the single molecular level is an exciting area of research. Dietz and Funke developed a V-shaped nanocaliper to study the nucleosome's bimolecular force (Figure 13a). The nanocaliper was integrated with two nucleosomes in a defined relative orientation, and its hinge angle can be used as a force spectrometer in a manner analogous to force spectroscopy. Following that, the hinge angle of the nanocaliper was used as an easily visible output to amplify nanoscale molecular interaction and motion. Using nanometer-resolution TEM images, we determined the energy landscape underlying the frequency at which a particular hinge angle occurs, as well as nucleosome-nucleosome distances. Castro and colleagues took a similar approach in developing a DNA origami-based nanocaliper to study chromatin rearrangement in the presence of target DNA (Figure 13b). They established that the nanocaliper angle is an accurate measure of nucleosomal DNA's end-to-end distance, allowing for the investigation of DNA protein interactions and chromatin conformational changes. Meanwhile, our group has integrated a pH-responsive Triplex DNA into this DMM to investigate pH variation on the nanoscale.

DMM has a high degree of structural rigidity and is easily visualized using direct imaging methods, such as AFM and TEM. It makes DMM uniquely suited for use in conjunction with high-resolution imaging techniques for understanding diverse subfields of biology at a single-molecule level. For instance, Zhuang et al. developed a fluorescent dye-labeled DNA origami rotor to quantify DNA rotation during various genome-processing reactions (Figure 14a). This DNA origami rotor featured four blades perpendicular to the rotation axis and a central dsDNA segment. The recBCD-induced unwinding of the DNA resulted in the spinning of the DNA origami rotor, which was tracked with millisecond time resolution at the single-molecule level. Moreover, using DNA origami as a "molecular landmark," our group deduced the molecular mechanism by which protein nucleators mediate the formation of fibrils in cells (Figure 14b). We decorated the triangular DNA origami with CsgB, a protein that directs origami rotor featured four blades perpendicular to the rotation axis and a central dsDNA segment. The recBCD-induced unwinding of the DNA resulted in the spinning of the DNA origami rotor, which was tracked with millisecond time resolution at the single-molecule level. Moreover, using DNA origami as a "molecular landmark," our group deduced the molecular mechanism by which protein nucleators mediate the formation of fibrils in cells (Figure 14b). We decorated the triangular DNA origami with CsgB, a protein that directs
and accelerates the self-assembly of the CsgA monomer’s fibril subunit. We confirmed that CsgB accelerated fibril formation, but we also observed fibrils growing out from or toward the CsgB at the DNA origami using high-speed AFM. In a similar fashion, the distance-dependent binding of immunoglobulin Gs (IgGs) to epitopes was demonstrated by capturing its transient conformations on a triangular DNA origami with a programmed spatial distribution of epitopes (Figure 14c).\textsuperscript{155} Thus, DNA nanotechnology’s ability to amplify biomolecular motions sheds light on the molecular mechanism underlying nanoscale interaction.

4.2. Biomolecular Factory

Combinatorial biosynthesis, such as mRNA-templated peptide synthesis during ribosomal translation, involves the use of protein or nucleic acid templates to direct reactions. This results in the selective generation of distinct multistep reaction products from a single set of substrates without the need for external intervention to modulate the effective molarities of substrates.\textsuperscript{152,153} However, the fabrication of nanoscale chemical species, such as nanoparticles and biomolecules, requires a continuous series of reactions. Specifically, due to thermodynamic and kinetic constraints, laboratory reactions for multistep synthetic products typically require multiple reactions using a variety of different substrates and reaction conditions. As they follow the track, DNA walkers change their physical location incrementally and autonomously over time. By simulating the biosynthesis process and protein, the programmable and controllable DNA nanostructure track can be used to template synthesize biomolecules.

The development of wholly synthetic DMMs with translational motion has been facilitated in particular by programmable DNA walkers with track lengths ranging from 20 nm to 1 \( \mu \)m, which provide an adequate track for multiple steps in synthesis. Moreover, the DNA motor can be programmed to transport a diverse range of chemical substrates, including RNA, aptamers, vaccines, and drugs. Thus, by combining the programming walking step with templated synthesis, these DMMs on the surface of the DNA nanostructures enable the walker’s arrival at each station to trigger a specific chemical reaction.\textsuperscript{154} Seeman et al., for example, demonstrate the use of a DNA walker to synthesize desired nanoscale chemical species of Au nanoparticles (Figure 15a).\textsuperscript{155} They connected individually selected nanoscale components in a stepwise and programmed manner on the track of a DNA origami surface. While the walker follows the track on the DNA nanostructure surface, the DNA motor encounters the three DNA devices sequentially and delivers its cargo to the desired location, promoting chemical reactions between attached moieties through proximity. Furthermore, Liu and He demonstrated an autonomous DNA walker capable of carrying out a series of amine acylation reactions while traversing a DNA track (Figure 15b).\textsuperscript{156} This system can synthesize multistep products at significantly higher yields than previous DNA-templated small molecule syntheses in a matter of hours.

It also has been demonstrated that DNA walkers regulate the function and performance of proteins and devices.\textsuperscript{159} Yang et al. and co-workers demonstrated a multienzyme synthetic system that enables precise and dynamic control of pathway activity (Figure 15c).\textsuperscript{158,159} They successfully and directly regulated the enzyme pathway with the aid of track by switching the active mediator between two enzyme pairs in a specific manner. Moreover, they demonstrated a novel photoresponsive DNA motor that converts energy light, a nonpolluting source of energy, to the motion of DNA through the incorporation of photoresponsive azobenzene molecules into DNA strands. The substrate channeling position and the interenzyme distance, which can be precisely controlled with light, dominated the performance of the enzyme cascade in this DNA walker.

![Figure 15. Autonomous synthesis with a DNA walker. (a) A DNA walker with "robotic arms" is capable of picking up different gold nanoparticles when traversing a programmed track with cargo-donating stations. AFM images show the change in nanoparticle position along the programmed track. (b) Sophisticated example of DNA-templated multistep synthesis programmed by a molecular walker. A DNAzyme-based walker modified with a primary amine is able to react with succinimidyl ester functionalized footholds to yield complex molecules. Reproduced with permission from ref 156. Copyright 2010, Nature Publishing Group. (c) Design and characterization of the enzyme pathway regulation system on DNA origami. Reproduced from ref 158. Copyright 2018, American Chemical Society.](https://jacsau.cdn.sciencemag.org/content/jacsau/2/15/2381.full.pdf)
breadboard or scaffold. DMMs can be programmed and integrated into a variety of algorithms via a probe connected to the DNA nanostructure interface. Turberfield et al., for example, demonstrated the integration of long-range communication and information processing using a synthetic DNA-based system (Figure 16a).\textsuperscript{165} Moreover, Seelig et al. demonstrated that signal propagation transports across the logic gates and signal transmission lines on DNA origami.\textsuperscript{166}

The path taken by a motor through a network of tracks with four possible routes can be programmed either externally or internally via instructions carried by the motor in this DNA walker.

The integrated synthetic DNA walkers enable the development of molecular computing networks capable of sorting and processing cargo in accordance with the instructions carried by them. Qian et al. demonstrated this DNA walker using a computing network (Figure 16b).\textsuperscript{167} They developed a simple algorithm for sorting cargo in a DNA robot by adding a new building block for leaving pheromone-like signals along a path. DMMs, in particular, could be programmed to take the shortest path possible and efficiently transport cargo molecules. With more effort directed toward the development of modular and collective molecular robots, as well as simple and systematic approaches, molecular DNA walkers could eventually be easily programmed in the same way that macroscopic robots are, but for use in microscopic environments. Our group extended this work by developing a DNA motor system capable of making decisions and performing other types of information processing (Figure 16c).\textsuperscript{168} Specifically, this DNA walker employed the progression of hybridization chain reactions to perform a parallel depth-first search on a ten-vertex rooted tree defined on a 2D DNA nanostructure surface (HCR). Notably, the pathfinding mechanism of this DNA motor is based on a localized strand exchange cascade that initiates at a unique trigger site on the origami and proceeds automatically along paths defined by DNA hairpins containing a universal traversal sequence.

4.4. Biosensing

It has been demonstrated that incorporating stimulus-responsive functional molecules onto DMMs results in the formation of desirable DMMs capable of functioning in a living system.\textsuperscript{169−172} A specific stimulus can be applied as an input to the DMM to cause it to switch to a reconfiguration and function that have been predefined. These programmable dynamic DMMs can be used in biological solutions and living cells as sensors and nanomedicine. For example, Funck et al. developed a cross-shaped DNA origami containing chiral plasmonic signals for the detection of viral RNA (Figure 17a).\textsuperscript{173,174} The DNA origami was constructed using two DNA origami arms and a gold nanorod with a strong circular arrangement was attached to each arm. The addition of target nucleic acid sequences altered the configuration of DMM via TMSD, with modified gold nanorods arresting the DMM in the defined chiral state. Meanwhile, Douglas et al. developed a robotic boxlike DNA origami capable of transporting molecular payloads to the cell surface in response to a defined stimulus.\textsuperscript{175} The DNA origami is a hexagonal barrel formed by attaching two domains of single-stranded scaffold hinges at the rear. To secure the barrel noncovalently, an aptamer-based lock was integrated into the front of the stapes. When the aptamer-based locks were switched to an open state in response to specific antigens on the cell surface, the barrel would expose the inner payloads. Moreover, by tethering two gold nanorods (AuNRs) to each surface of two origami bundles with a reconfigurable DNA framework, Liu et al. created a reconfigurable 3D plasmonic rotary motor, which executes DNA-regulated conformational changes at the nanoscale (Figure 17b).\textsuperscript{176} Specifically, the addition of fuel DNA drives the metamolecules to distinct conformational states, resulting in transducing their conformational changes in situ into circular dichroism changes in the visible wavelength range simultaneously.

In 2009, Krishnan et al. developed an i-motif DNA-based DMM that functions as a pH sensor for monitoring endosome maturation within living cells, representing the first instance of DMMs on the surface membrane being used in vivo.\textsuperscript{177} Due to
the DMM’s biocompatibility and byproducts, they discovered that it had a negligible effect on processivity. They then extended the DMM to map enzymatic activity and membrane potential in intracellular organelles, demonstrating DMM’s universality in sensing and diagnostics in living systems.\textsuperscript{178–183}

In addition, given the tremendous potential of functional AuNP-based, SNA (spherical nucleic acid) for imaging, diagnosis, and treatment of disease, the incorporation of DMM onto nanoparticles enables the development of smart DMM systems that can change their properties in response to biological stimuli.\textsuperscript{184,185} For example, the DNA monolayer formed on the surface of the AuNPs controls the configuration of AuNP assemblies, allowing to assemble shape-shifting molecular machines with controlled biological functions. More importantly, the combination of DMMs and nanoparticles enables endocytosis of the DNA motor-AuNP complex. This synergistic effect enables these DMMs to function within the living cell, enabling to detect living biomolecules rapidly and sensitively. For instance, Le et al. successfully constructed a DNAzyme motor that anchors to the surface of a nanoparticle in response to a specific intracellular target (Figure 18a).\textsuperscript{186} Moreover, Pei et al. further demonstrated that exonuclease III, like DNA, can also autonomously move on DNA tracks on nanoparticle surfaces (Figure 18b).\textsuperscript{187} Particularly, they further confirmed that the performance of the DNA walker is critically dependent on the DNA density and the track conformation by interrogating the morphological effect of the 3D track on the nuclease activity. Intracellular interaction between a target molecule and the motor system initiates the motor’s autonomous walking on the AuNP, allowing for amplified detection of the specific microRNA in individual cancer cells.

The integration of therapeutic components into the DMM enables the development of intelligent therapeutic carriers and sensors with enhanced in vivo reconfigurability. Ding et al. demonstrated a thrombin-activated DMM for cancer therapy via a vessel blockade (Figure 19a).\textsuperscript{188} In the DMM, thrombin was captured by extending strands from a specific location on a rectangular DNA origami sheet. The origami was then further assembled into a tubular shape using an aptamer that acts as a molecular lock for nucleolin, effectively encapsulating the thrombin within its inner cavity. When the DMM is delivered to cancer sites, the aptamer locks interact with the nucleolin expressed by tumor-associated endothelial cells, altering its configuration and thus inhibiting tubular origami. This rendered the encapsulated thrombin capable of inducing blood coagulation via thrombosis, resulting in tumor necrosis and inhibition of tumor growth due to a lack of oxygen and nutrients from blood vessels.

They also used a similar method to construct a DMM for cancer vaccine therapy.\textsuperscript{189} The DMM used peptides as antigens, CpG DNA as a nucleic acid adjuvant, and triplex DNA as a molecular lock, rather than thrombin. When the mildly acidic intracellular environment of lysosomes in antigen-presenting cells is stimulated, the molecular locks of triplex DNA unfasten, exposing adjuvants and antigens for activation of a robust immune response. These DMMs served as a model for the development of intelligent nanoplatforms for biosensing and nanomedicine applications.\textsuperscript{190–194}

Furthermore, the DMMs could be used as a novel therapeutic nanomedicine.\textsuperscript{195,196} Their therapeutic properties and biocompatibility, in particular, enable a programmable approach to medical therapies. For example, our group discovered that intact folding of DNA origami nanostructures larger than 100 nm resulted in their preferential accumulation in the kidneys of mice rather than their biodegradation via liver uptake (Figure 19b).\textsuperscript{197} We also demonstrated that these DNA frameworks have an active ROS scavenging effect in vivo, alleviating AKI-related clinical symptoms. Furthermore, Ge et al. modified a rectangular DNA origami nanostructure with a 3aC5a aptamer to create DMM for the exclusive retention of kidneys from oxidative stress-induced AKI.\textsuperscript{198} Since the 3aC5a aptamer inhibits C5a-mediated inflammation, the renally accumulating DMM may suppress inflammatory responses when the kidney retention time is prolonged (>12 h). As a result, DMMs are attractive candidates for the treatment of acute kidney injury associated with renal diseases.

5. CONCLUSIONS AND OUTLOOK

In summary, a variety of functional DNA molecules and nanostructures have been successfully used to construct complex and sophisticated DMMs.\textsuperscript{199–201} The advancement of DNA nanotechnology promotes the development of DNA walkers, motors, rotors, sensors, and robots. Moreover, the development of DMMs aids in the comprehension of physical theories of transport phenomena and aids in the clarification of the relationship between mechanical motions and chemical reactions. We have discussed recent advances in how integrating DMMs into various interfaces can help to empower their functions and applications in this Perspective. For nucleic acid chemistry, an increasing number of moieties and functional molecules have been developed, allowing for easy assembly and integration of diverse functions.\textsuperscript{7} The versatile
intelligent DMMs were developed for a variety of biological applications, including biomolecular rulers, biosynthesis, biocomputing, and biosensing. One of the most significant challenges for artificial DMMs is to approach the complexity and intricacy of natural proteins. Since current methods focus on substituting allosteric and mechano-gating coupling into DNA nanostructures, engineering mechanochemical coupling requires a diverse set of fully predictable monomeric components. The highly precise discrete DNA nanostructures created by DNA oligomers or DNA origami provide an extremely useful method for guiding motion propagation. Nanometer-scale precision in molecule arrangement via DNA hybridization, in particular, enables the modification of DMMs with a variety of functions. Meanwhile, the inability to program DMM energy landscapes restricts the field of intelligent and efficient molecular machines. Later, as mass production of DNA and scaled-up DNA self-assembly technologies are developed, the cost and difficulty of building DMMs will decrease, thereby expanding the area of their biologic applications. Thus far, the development of integrating DMMs at the interface has paved the way for a sophisticated approach to the construction of artificial DMMs with complex structures and functions.

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**Notes**

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