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Review

Self-assembly of DNA origami for nanofabrication, biosensing, drug delivery, and computational storage

Zhimei He,[1,2,3] Kejun Shi,[1,3] Jinggang Li,[1] and Jie Chao[1,2,*]

SUMMARY

Since the pioneering work of immobile DNA Holliday junction by Ned Seeman in the early 1980s, the past few decades have witnessed the development of DNA nanotechnology. In particular, DNA origami has pushed the field of DNA nanotechnology to a new level. It obeys the strict Watson-Crick base pairing principle to create intricate structures with nanoscale accuracy, which greatly enriches the complexity, dimension, and functionality of DNA nanostructures. Benefiting from its high programmability and addressability, DNA origami has emerged as versatile nanomachines for transportation, sensing, and computing. This review will briefly summarize the recent progress of DNA origami, two-dimensional pattern, and three-dimensional assembly based on DNA origami, followed by introduction of its application in nanofabrication, biosensing, drug delivery, and computational storage. The prospects and challenges of assembly and application of DNA origami are also discussed.

INTRODUCTION

Molecular self-assembly plays an indispensable role in biology, chemistry, and materials. The self-assembly process is usually accomplished by a combination of weak interactions (e.g., hydrogen bonding, van der Waals, and hydrophobic interaction). At present, a variety of molecules have emerged as primary self-assembly units, such as DNA,[1] RNA,[2,3] protein,[4] lipid,[5] organic molecule,[6] etc. Among them, DNA as an inherently biocompatible material is widely used in molecular self-assembly due to its unique properties (e.g., biocompatibility, high specificity, and Watson-Crick base pairing). Inspired by the naturally occurring Holliday junction structure,[7] Seeman et al. encoded immobile Holliday junction structure based on minimization of sequence symmetry, which laid the foundation for the structural DNA nanotechnology.[7–9] Since then, DNA nanotechnology has opened up a new era.

DNA is a macromolecular polymer composed of four bases of adenine (A), thymine (T), cytosine (C), and guanine (G), deoxyribose, and phosphoric acid. According to the Watson-Crick base pairing principle, A binds to T with two hydrogen bonds while G binds to C with three hydrogen bonds. The number and order of bases in the polynucleotide chain determine the coding information and structural function of DNA.[10] Through this specific and highly predictable pairing rule, the interaction between DNA oligonucleotides can be well programmed. And the convenient synthesis of DNA greatly promoted the development of DNA nanotechnology. And the manufacture of DNA nanostructures is divided into top-down and bottom-up methods. The bottom-up approach is to integrate small, simple DNA structures into large-scale, complex structures, typically through molecule-molecule recognition, which allows programmable and predictable DNA assembly. The top-down approach is to reduce the size of large, complex DNA structures to desired size or pattern through external assembly tools. DNA tile-based self-assembly adopts bottom-up strategy and is an important part of the evolution of DNA nanotechnology. The basic structures, ranging from initial Holliday junction to DX (double crossover),[11] TX (triple crossover),[12] PX (paranemic crossover),[13] JX2 (paranemic crossover with two juxtaposed sites)[13] to 6HB (six-helix bundles),[14] can be assembled into structures of different dimensions by virtue of the sticky end. As a unique bottom-up self-assembly nanotechnology, DNA origami was first proposed by Rothumend in 2006.[15] It builds large, arbitrarily designed DNA nanostructures by folding a long scaffold DNA with hundreds of staple strands in one step. Later, this technology was utilized to create a DNA pattern resembling the map of China,[16] DNA origami boxes,[17] and highly complex two-dimensional and three-dimensional DNA origami structures.[2,10–13,18] Due to its strict

[1]Key Laboratory for Organic Electronics & Information Displays (KLOEID), Jiangsu Key Laboratory for Biosensors Institute of Advanced Materials (IAM) and School of Materials Science and Engineering, Nanjing University of Posts & Telecommunications, Nanjing 210023, China

[2]Smart Health Big Data Analysis and Location Services Engineering Research Center of Jiangsu Province, School of Geographic and Biologic Information, Nanjing University of Posts & Telecommunications, Nanjing 210023, China

[3]These authors contributed equally

*Correspondence: iamjchao@njupt.edu.cn

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base pairing, programmability, and full addressability, DNA origami has become as an emerging technique to construct highly complex two-dimensional patterns and three-dimensional nanostructures, opening new horizons for fabrication of well-defined nanoassemblies. Combining the advantages of bottom-up DNA self-assembly and top-down lithography may offer a practical approach for fabrication of electronic and photonic devices with nanoscale precision. Unlike the bottom-up approach, a top-down, automatic, geometry-triggered sequence design strategy was proposed to program customized DNA origami structures only according to the target geometry.[19]

Given that the performance of nanomaterials largely depends on their dimension, shape, and composition, it is very important to precisely manipulate the morphology of nanostructures in nanoscience. Firstly, DNA origami provides a straightforward technology to fabricate complex structures with nanoscale accuracy and remarkable addressability that allows for the prescribed distribution of different elements on DNA origami with nanoscale precision.[14,18] Therefore, DNA origami affords a potent tool for customizing the morphology and functions of diverse nanomaterials. The assembly of nanomaterials with well-defined morphology based on DNA origami-supported template has attracted the attention of researchers.[15,16] Secondly, DNA nanostructures exhibit inherent biocompatibility, high tissue penetration capacity, and sufficient biostability, which is conducive to their biomedical applications.[20] Thirdly, strict Watson-Crick base pairing rules enable the integration of functional nucleic acids (e.g., aptamers, DNAzymes, and i-motifs) into DNA structures. An excellent drug delivery system is expected to maximize the therapeutic efficacy of drugs with minimized off-target side effects. Recent preclinical applications of DNA origamis as nanovehicles for therapeutic cargo delivery highlight their potential as potent alternatives to traditional nanocarriers, which benefits from the inherent advantages of DNA origamis (e.g., biocompatibility and precise spatial addressability).[17,21,22] By integrating the targeting aptamer and response units into the design of nanocarriers, a targeted and smart nanosystem based on DNA origami is successfully fabricated. The prominent merits of DNA nanovehicles are improved delivery of hydrophobic drugs, enhanced biostability, targeted drug delivery, enhanced delivery across biological barriers, and improved pharmacokinetics. In addition, DNA-based storage technology outperforms most traditional storage media in terms of physical density, information retention time, and volumetric coding capacity.[23,24] Advances in DNA synthesis and sequencing technique have laid the foundation for construction of large DNA databases with powerful storage capacity and non-destructive data recovery. Several potent DNA storage structures capable of rewriting, random access and error correction have been created, showing potential for cost reduction and scalability.[19,25,26] In addition to information storage, DNA origami can also serve as a multifunctional molecular computing substrate. In general, attributing to the intrinsic advantages of addressability and shape controllability, DNA origami has been widely used in many fields. This review will focus on DNA origami, the corresponding two-dimensional and three-dimensional assembly, as well as its application in nanofabrication, biosensor, drug delivery, and computational storage (Figure 1).

ASSEMBLY OF DNA ORIGAMI

DNA origami

DNA origami is a technology of molecular self-folding that folds a long single-stranded DNA scaffold (typically M13 bacteriophage genome DNA) into a well-defined object by hundreds of short-stranded DNA staples in a one-pot process. As driven by Watson-Crick base pairing of double-stranded DNA (dsDNA) hybridization, a unique set of DNA staples are supplemented in different parts of the long single-stranded DNA scaffold without the use of restriction enzymes or DNA ligases, endowing DNA origami intrinsic addressability. The concept of DNA origami was pioneered by Rothemund in 2006.[15] By taking advantage of DNA origami technology, he constructed a series of two-dimensional DNA nanostructures such as triangles, pentagram stars, and smiling faces. Such assembled DNA origamis (~100 nm) exhibited programmable patterns with a spatial resolution of ~6 nm, enabling engineering of more intricate or larger structures. Firstly, by virtue of bottom-up DNA origami technique, DNA structures are no longer limited to some simple and regular geometric patterns. Large-scale, structurally stable, and arbitrary DNA shapes could be built, which compensates the poor size control faced by DNA tiles. Secondly, M13 bacteriophage genome DNA is the most commonly used scaffold for assembly of DNA origami and the corresponding staple sequences can be automatically designed by software (e.g., caDNAno)[27] according to the routing pathway. Finally, the assembly of DNA origami has no strict requirement on the stoichiometric ratio and purity of DNA staple strands. These above advantages make DNA origami a powerful tool which promotes the prosperity and development of DNA nanotechnology. In the same year, researchers applied DNA origami to create an asymmetrical complicated DNA nanostructure resembling China map with a diameter

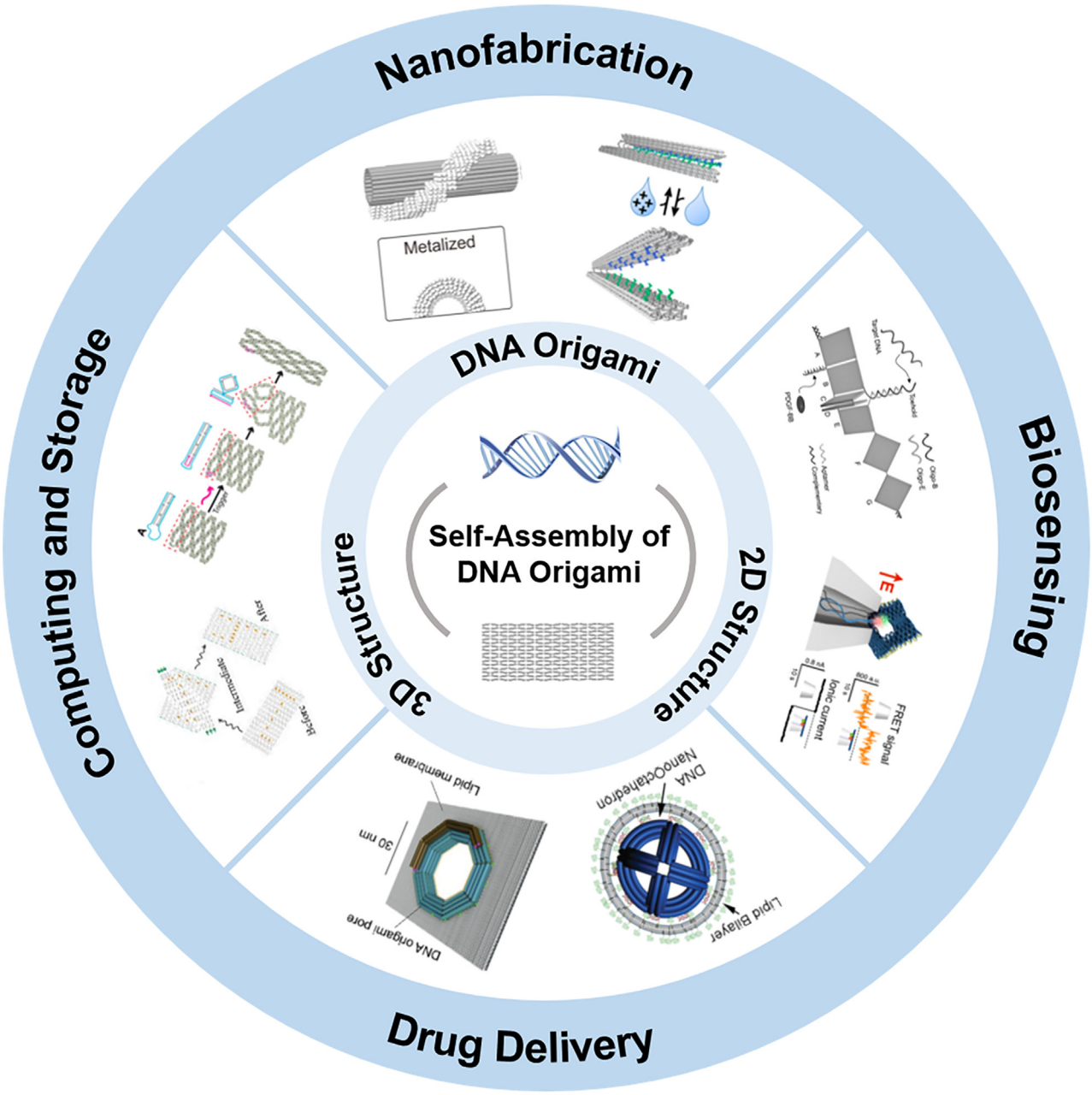

Figure 1. DNA nanostructure-enabled applications

DNA origami, 2D and 3D DNA structures and their applications in nanofabrication, biosensing, drug delivery, and DNA computing and storage.

of about 150 nm, demonstrating the potent capacity of DNA origami in constructing complicated and exquisite patterns.[16] In 2008, Andersen et al. developed an interactive software package to guide the formation of DNA origami structures (Figure 2A).[28] This software package enables automatic generation of complex DNA origamis and manual editing, and demonstrates its applicability by designing of asymmetric dolphin-like DNA origami structures as observed by high-resolution atomic force microscopy (AFM) imaging in liquid. The flexibility of the dolphin tail is well controlled by adjusting the number of insertion points in the tail region, the direction of the origami, the tip of the AFM probe, and the magnitude of the applied force on the tip of the AFM probe. In 2009, researchers engineered a closed tetrahedral cavity based on DNA origami (Figure 2B).[29] In this work, DNA scaffold strand, which runs through the whole structure, is fixed by numerous staple chains to create four triangular objects that are bridged together by the

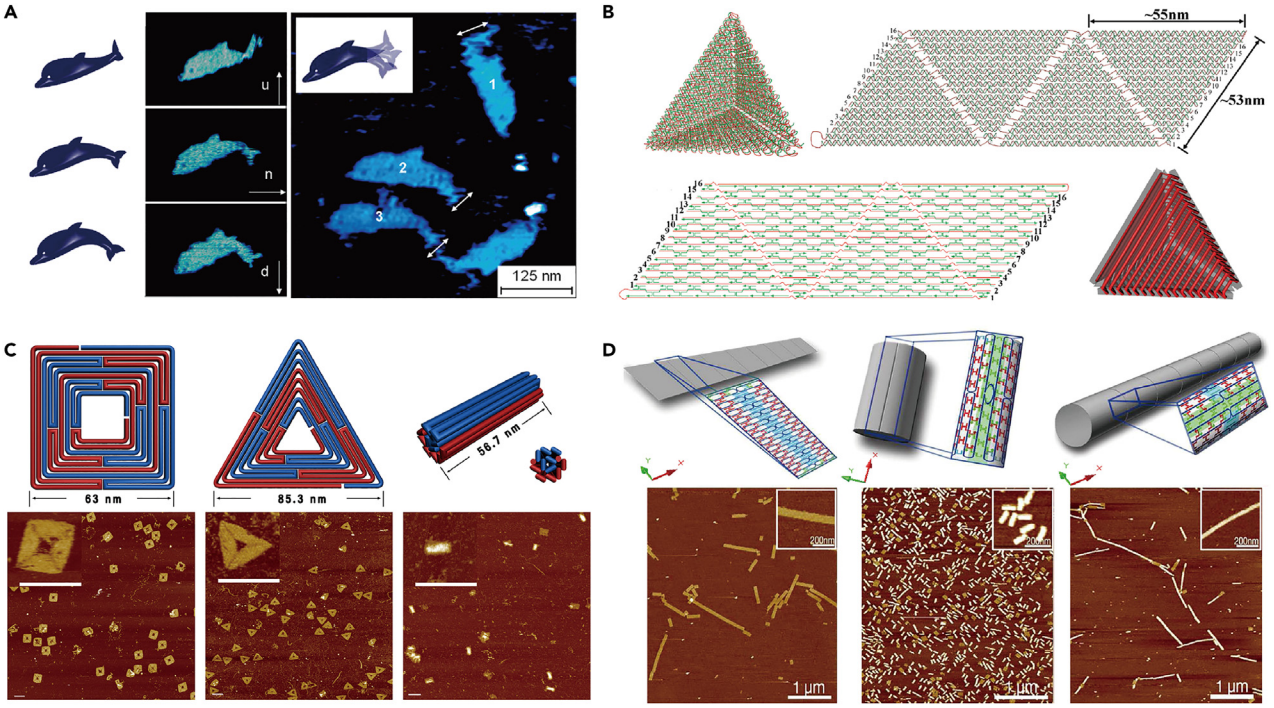

Figure 2. The construction of intricate DNA origamis

(A) AFM images of dolphin-shaped DNA structures with different conformations.[28] Copyright 2008 American Chemical Society.

(B) The 2D schematic and 3D model of the DNA tetrahedron composed of closed triangles.[29] Copyright 2009 American Chemical Society.

(C) Complete square, triangular, and 24HB DNA origami structures assembled from a dsDNA scaffold.[34] Scale bars: 100 nm. Copyright 2012 American Chemical Society.

(D) The schematic and the corresponding AFM images of DNA origami-based nanoribbons and nanotubes.[35] Copyright 2012 American Chemical Society.

successive scaffold. The cavity in this structure enables the DNA tetrahedron to package proteins, metal nanoparticles, etc., which can be expanded for site-specific functionalization or targeted cargo delivery. Subsequently, Andersen et al. constructed an addressable 3D DNA box with a dimension of 42 × 36 × 36 nm.[17] Six DNA origami regions were obtained by pairing a scaffold strand with numerous staple strands and then a 3D box structure was created by virtue of secondary annealing. By introducing the lock–key concept, this work also demonstrates the dynamic control and programmability of the DNA lid, opening new horizons for logic sensors and on-demand cargo release. However, the opening mechanism of the lid is an irreversible process, so it is difficult to realize open/close multiple switching. In order to solve this problem, researchers constructed a smaller DNA box capable of reversible reconfiguration.[24] This formed DNA box is structurally reconfigurable and can be opened and closed multiple times as triggered by the externally imposed keys. In 2010, researchers demonstrated the versatility of DNA fold-and-cut technique in programming reconfigurable buildings by taking Möbius strip as the demonstration target.[30] Benefiting from the strand displacement technique, DNA origami could be reconfigured into supercoiled structures and ring structures, which enriches the topological diversity of 2D or 3D DNA origamis. Due to the structural complexity and numerous components involved, the design and construction of customized DNA origamis may be a major obstacle for novices in this field. In 2011, a computational tool called computer-aided engineering for DNA origami (CanDo) was designed based on the open-source caDNAno design files, which can model the 3D structure of DNA origami, predict the flexibility of DNA origami, and point out the conditions for DNA origami to maintain the structural integrity.[31] It offers a user-friendly interface that guides scientists without prior training in DNA origami technology to engineer customized DNA origamis for specific purposes. Subsequently, the same group extended and systematically demonstrated the computational modeling framework CanDo.[32] Apart from predicting the solution shape and flexibility of DNA origami, this modeling framework can also reveal the delicate structural characteristics (e.g., global out-of-plane deformations) of DNA origamis, providing a powerful tool for the development of DNA nanotechnology. In 2012, researchers synthesized a 26 kb long-chain DNA scaffold using long-range PCR technique followed by enzymatic digestion, which could be folded into super-sized rectangle

DNA origami (238 × 108 nm) by nearly 800 32-base short strands.[33] It provides a strategy for synthesizing longer DNA scaffold as a complement to M13 bacteriophage genome DNA to form super-sized DNA origami. Researchers artificially amplified the size of DNA structures using dsDNA scaffolds to directly construct DNA origami that involves the two constituent single-stranded DNA (ssDNA) fragments (Figure 2C).[34] In contrast to the previously reported strategy in which dsDNA was entirely divided into two independent ssDNAs that were further isolated by excess staple strands, this work relies in part on cooperation between the two constituent ssDNA fragments to form an integrated object. It can assemble a triangular structure with larger size and stronger rigidity, realize the splicing between triangles and squares, and even form a five-element square lattice structure. However, this method cannot completely assemble large DNA origami with flexible junctions, so it still remains challenging to scale up the assembly of DNA origami using ultralong dsDNA templates. In 2013, researchers reported a one-step assembly of one-dimensional nanotubes and nanoribbons based on DNA origami with precise control over width and diameter (Figure 2D).[35] Benefiting from the full addressability of DNA origami, proteins could be accurately anchored on the formed DNA nanostructures with a resolution of less than 10 nm and at the single-molecule level. A pair of enzymes are connected to the interior of the resultant DNA nanotubes which functions as confined nanoreactors to accommodate enzymes so as to achieve high coupled enzyme cascade. In 2017, researchers proposed a framework for assembling an individual DNA or RNA strand into a complex but unknotted single-stranded origami (ssOrigami) structure.[2] This ssOrigami does not involve auxiliary strands and can be assembled into diverse spatially compact structures. In addition, the authors have demonstrated that the chain could be easily replicated *in vitro* and in living cells. In 2018, researchers explored the stability of three kinds of DNA origamis namely triangular, 24-helix bundle (24HB) and 6-helix bundle (6HB) in low magnesium buffers and found that 6HBs were more tolerant to low Mg^{2+} condition.[36] The presence of EDTA and phosphate ions may promote DNA origami denaturation likely by replacing magnesium ions on the DNA backbone and lowering the interaction between magnesium ions and DNA, respectively. DNA nanostructures require solution with a high ionic strength to remain intact, which in turn introduces surface charges that impedes material deposition, so it is still challenging to fully exploit the potential of DNA nanostructures. To address this problem, researchers reported a modified STöber method for preparing biomimetic silica nanostructures on DNA origami-supported templates.[37] Framework-like, curved and porous DNA nanostructures were employed to illustrate this method. In addition, the thickness of the deposited amorphous silica layer can be tuned by adjusting the growth time, and the DNA–silica hybrid framework is tougher than the DNA origami template while retaining flexibility, affording a general approach for constructing bionic silica nanostructures. In 2020, researchers demonstrated the steganography strategy based on molecular information coding in a DNA origami domino array (DODA) that can reconfigure the intrinsic modes while keeping the morphology (size and shape) of DODA unchanged.[38] In the same year, the same team also proposed a DODA-based dynamic mode operation system (DODA DPO). Under the stimuli of a set of trigger strands, a spontaneous stacking conformation cascade from the "before" to the "after" conformation is presented. Conformational transformation further pulls the operation mode units closer to trigger DNA strand displacement cascade so as to perform three different mode operations of writing, erasing, and shifting.[39]

Two-dimensional assembly based on DNA origami

Since the size of a single DNA origami is limited, two-dimensional assembly is used to enlarge the scale. In 2006, researchers reported a facile strategy for assembling ssDNA into the desired 2D pattern.[15] The staple chains at the edge portions are paired by complementary bases to yield a tunable combination of shapes, avoiding aggregation at high concentration by introducing hairpin structures. This idea laid the foundation for the assembly of DNA origami. With the further development of DNA origami technology, it has become an urgent need to expand the topological scale of DNA origami structure. In this context, researchers proposed a method to engineer large-scale 2D DNA origami using rectangular DNA tiles as staple tiles instead of using conventional staple chains (Figure 3A).[40] This strategy can be applied to fabricate large-scale DNA origami objects with the size range comparable to traditional photolithography, thereby bridging bottom-up assembly with top-down photolithography. Subsequently, this research team improved and extended the previous method. First, a ssDNA scaffold was pre-folded into a loose frame as fixed by a set of bridge strands, and then the pre-made single DNA origami tiles were directionally assembled onto the frame (Figure 3B).[41] The size of the DNA structure assembled by this method is increased by an order of magnitude as compared with a single DNA origami tile unit, which holds great promise in building greater spatially addressable architectures. In 2011, researchers organized DNA double-crossover tile to yield a two-dimensional array with an edge size of 2–3 μm, which seems large enough to bridge bottom-up patterning methods with top-down

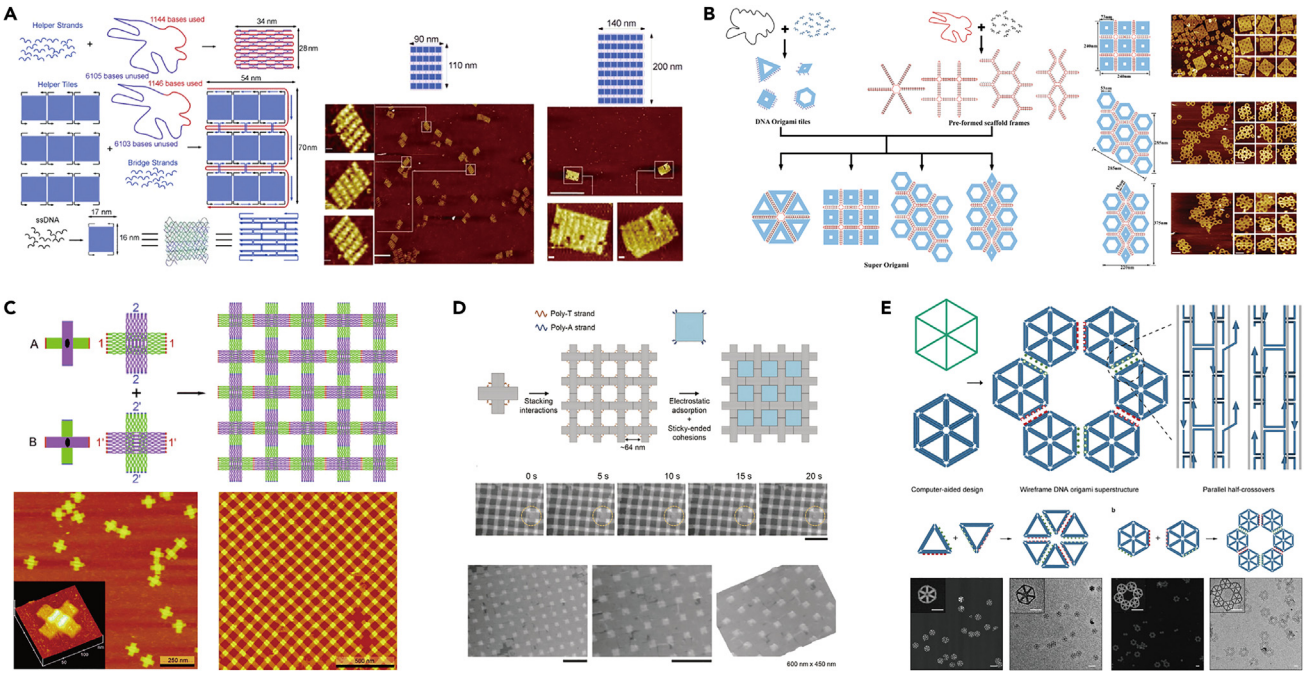

Figure 3. 2D DNA origami structures

(A) The construction of higher-order origami structure by taking nine 8-helix tiles as staples to fold M13 bacteriophage genome DNA scaffold.[40] Scale bars for the insets are 20 nm. Copyright 2010 John Wiley and Sons.

(B) Schematic illustrating the assembly process of DNA superstructures composed of origami tiles and pre-designed scaffold frames, and the corresponding AFM images.[41] Scale bars : 200 nm for zoom out images and 100 nm for zoom in images. Copyright 2011 American Chemical Society.

(C) Schematic illustration of the two-dimensional array organized by two distinct tiles (A tile and B tile) and AFM image of A tile.[42] Copyright 2011 John Wiley and Sons.

(D) Schematic showing the trapping of square-like origamis into the cavity of a preformed DNA framework and the corresponding AFM images.[54] Scale bars: 200 nm. Copyright John Wiley and Sons.

(E) Schematic representation, AFM and TEM images of super-sized DNA structures assembled from METIS DNA origami.[59] Scale bars: 100 nm. Copyright 2022 American Chemical Society.

methods (Figure 3C).[42] Subsequently, researchers programmed the geometric arrangement of DNA origami based on the blunt-end stacking interaction, and demonstrated that both binary code and shape code can function as the basis of these stacking bonds.[43] A chain consisting of five rectangle DNA origami was assembled with different types of orthogonal stacking bonds. Compared to DNA hybridization, this method allows for easier reprogramming of bond types after synthesis and does not need purification commonly required in the sticky end-based method. In 2014, researchers discovered that monovalent cations can mediate the adhesion and mobility of the DNA origami on the surface of mica.[44] Lattices can be formed either by blunt-end stacking between origamis or tight packing of symmetric origamis, affording a template for ordered anchoring of proteins. Subsequently, researchers also organized rectangular DNA origami into a two-dimensional lattice by means of programmed control of surface diffusion realized by varying the concentration of surface cations.[45] In addition, this work shows that the model of dsDNA cannot be simply converted to DNA nanoarchitectures due to the influence of the size and shape of DNA on the binding of DNA to mica. Therefore, it is necessary to establish a comprehensive theory of DNA nanostructures that covers structural geometry, cation type, and protocols of surface treatment. In 2015, researchers proposed a strategy for preparing wireline-frame DNA patterns in which the lines represent varying-length antiparallel DNA crossover tiles and the vertexes are denoted by angle-controlled multi-arm junctions (n × 4, n = 2–10). DNA scaffolds were utilized to integrate the lines and vertexes into well-defined nanostructures with highly programmability.[46] Unfortunately, the misfolding of origami will discount the yield and sometimes the misfolded structure cannot be isolated from the desired structure. Subsequently, researchers constructed a DNA origami system capable of forming a series of fully addressable objects that represent the discrete and approximately degenerate energy minimum in the huge folding events.[47] The high yield of well-assembled DNA origamis demonstrates the existence of effective assembly paths. Then, researchers constructed two types of cholesterol-modified DNA origamis (a Y-shaped DNA nanostructure and a three-layer rectangular object) to mimic

membrane-mediated organization into hierarchical superstructures on lipid bilayers and monolayer vesicles.[48] The addition of different poly-oligonucleotides to the origami blocks anchored onto the bilayer gives rise to the generation of linear polymers or 2D lattices on the lipid bilayer. This study offers the possibility in fabrication of DNA architectures resembling the membranal clathrin or caveolin that play a vital role in the endocytosis. In the same year, researchers proposed a method for organization of 2D DNA origami lattices with the aid of lipid bilayer.[49] Interestingly, the mica-supported zwitterionic lipid bilayer functions as a platform to enable the electrostatic attachment and self-assembly of DNA origamis in the buffer solutions used to fabricate DNA origamis. Moreover, even without any buffer exchange process, pre-folded DNA origami blocks can be easily deposited on the lipid bilayer to assemble into periodic origami lattices. In 2016, researchers constructed a series of DNA origami-based hexagonal tiles (HTs) that were programmed into honeycomb lattices to generate tubular or planar lattices.[50] Benefiting from the elaborate design and computational modeling feedback, the mechanical properties of origami tiles and the interconnection between tiles are well controlled. In addition, HTs and lattices were proved to be capable of constructing plasmonic metamaterials through the well-defined arrangement of gold nanoparticles (AuNPs). Subsequently, researchers proposed a framework-mediated assembly based on DNA origami that tackles the challenge facing two-dimensional assembly of amphiphilic molecules in aqueous solutions.[51] The resultant nanosheets of amphiphilic molecules can be isolated from the DNA origami by discrete DNA double strands to minimize the effect of the substrate while maintaining the impermeability and mobility of the amphiphilic layer. Particularly, the distance between DNA origami and nanosheets can be well controlled by precisely tuning the number of base pair in the DNA anchor chain. In 2017, researchers reported a versatile strategy for building large-scale DNA relay arrays with interconnected modular building blocks.[52] Each block represents a dynamic unit capable of transmitting its structural information to adjacent block, and the study of DNA relay arrays provides an approach for programmable, multistep, and long-range reconfiguration of DNA arrays. To overcome the limitations of DNA origami in integrating more traditional patterning methods and larger layouts, researchers proposed a method termed "fractal assembly" that follows straightforward local assembly rules.[53] They recursively modified and applied these rules in a hierarchical, multi-stage assembly process in which a small group of constant unique DNA strands is employed to create DNA origami arrays with increasing size and well-defined patterns (e.g., Mona Lisa and Rooster). In 2018, researchers employed a "complementary" origami structure with a dimension fitting the inner cavity of the planar frame to enrich the patterns of a pre-organized planar origami frame (Figure 3D).[54] This 2D framework is formed by stacking or sticky-ended cohesions of cross-shaped origami distributed over a mica-supported lipid bilayer. This sequential self-assembly method paves the way for construction of higher-order origami structures. To reveal the reason for the low yield of origami polymers, researchers performed a series of kinetic studies on the rectangular origami dimerization reaction and the origami dimer dissociation reaction using the single-molecule fluorescence technique.[55] According to the reaction kinetics/yields obtained at different conditions with varying origami concentrations, ion concentrations, ion types, bridge chain lengths, and assembly strategy, the self-dimerization is believed to be responsible for the limited production of desired dimers, likely through the blunt-end interactions caused by the bridging chains. Such non-specific dimerization can be inhibited by controlling the base pair number of bridging chains, edge design, and ion concentration. In 2019, Zhang et al. programmed two-dimensional DNA origami into multiple higher-order configurations by virtue of hydrophobic interactions, providing an efficient strategy for building diverse nanoarchitectures from the same DNA origami.[56] Subsequently, researchers proposed a fully autonomous program to design the sequences of staple strands required to assemble arbitrary planar DNA origami wireframe structure.[57] Dual-duplex edges coupled with a dual graph scaffold routing program ensure the programming of 2D DNA origamis with arbitrary edge length, vertex degree, and vertex angle while maintaining the structural integrity. In the same year, the same team presented a computational method for programming two-dimensional origami wireframe assemblies with honeycomb edges made up of six parallel duplexes.[58] Compared with the previous dual-duplex counterparts, the mechanical rigidity of the honeycomb-based two-dimensional origami design is enhanced. Given that the dimension of a DNA origami architecture is often restricted by the length of the DNA scaffold, researchers presented a hierarchical assembly approach combining lateral cohesive interaction with half-crossings to overcome this limitation by organizing wireframe DNA origami units into origami superstructures and even ordered arrays (Figure 3E).[59] The practicability of the strategy is demonstrated by producing dimers and higher-order hexahedral architectures based on triangular and hexagonal wireframe origami units.

Three-dimensional assembly based on DNA origami

Expanding the size of DNA origami through 3D assembly can broaden the application scope of DNA origami. Researchers developed a software package called caDNAno that guides the sequence design

for construction of 3D honeycomb assemblies.[27] The software package allows users to complete the conversion of the design to DNA sequences, which greatly reduces the work load required to design 3D DNA origami structure. Shawn Douglas et al. constructed a customized 3D origami lattice.[60] The feasibility of this design and assembly was demonstrated on six different shaped objects with sizes between 10 and 100 nm. In addition, the hierarchical assembly of stacked cross-polymers and heterotrimeric wireframe icosahedron is also demonstrated, which provides a promising strategy for hierarchical assembly of complicated architectures. Similarly, another team built a 3D origami structure with a more compact square lattice, enhancing the density of DNA helices but yielding flatter surfaces than honeycomb lattices, which provides a more natural framework for rectangular nanostructures (Figure 4A).[61] In 2012, the same team created the hexagonal-lattice close-packing for helices in 3D DNA origami, and presented a hybrid 3D DNA origami that integrates honeycomb-, square-, and hexagonal-lattice geometry (Figure 4B).[62] Compared with the previously reported structures, this design exhibits higher spiral packing density and higher spatial addressing resolution. Subsequently, researchers explored the potential of nanoscale distortion of complicate shapes in DNA design.[63] By deleting and inserting different numbers of base pairs at prescribed positions, DNA helical bundles can be distorted to varying degrees with controllable curvature. In addition, different curved structures are integrated to generate different types of sophisticated nanoarchitectures. Such programmable DNA bending structure may function as a probe to explore the binding tendency of proteins with the pre-bent DNA substrates. Unlike traditional rigid lattices, another team engineered DNA objects with complex curved surfaces, which introduces out-of-plane curvature by tuning the specific sites and modes of crossover between neighboring DNA duplex.[64] This strategy was extended to construct a series of DNA nanobuildings with high curvature (e.g., concentric rings, spherical shells, and vase). In 2014, researchers presented a facile and general approach for layered assembly of polyhedra (e.g., tetrahedrons, triangular prisms, cubes, pentagonal prisms, and hexagonal prisms) using a rigid three-armed DNA origami tile featuring inter-arm angle controlled and strengthened by supporting struts and vertex helices, respectively.[65] In 2015, researchers proposed a design strategy to prepare finite-size origami wireframe assemblies, and demonstrated its feasibility in fabrication of highly complicated and programmable 3D wireframe Archimedean solid structures.[46] Subsequently, researchers mimicked the mechanism by which ribonuclease P recognizes pre-transfer RNA by programming DNA to generate individual, complementary 3D DNA objects that interact through stacking interactions.[66] By taking advantage of this principle, homogeneous and heterogeneous DNA-polymerized lattices (e.g., single-strand and double-strand filaments, lattices at the micron scale) and reconfigurable devices (e.g., actuator, switchable gear, and foldable nanobook and nanorobot) were fabricated. Given that the general method and design software of DNA origami polygon mesh have limitations originating from DNA geometry and pairing, some manual adjustments are often required at the design stage. In this context, researchers proposed a general strategy for assembling arbitrary polygonal digital grids in DNA, which enables a highly automated design process to generate DNA structures that are difficult to implement using previously reported methods.[67] The scaffolded DNA origami method is restricted by the necessity to manually program specific Watson-Crick base pairing for desired structures. In 2016, researchers presented a completely automatic reverse design strategy, namely, DNA Origami Sequence Design Algorithm for User-defined Structures, which utilizes the input wireframe grid to produce any wireframe DNA components without relying on user feedback or restrictions on spherical topologies.[19] The strategy was employed to design 35 kinds of Platonic, Archimedean, Johnson and Catalan solids, 6 asymmetric objects, and 4 polyhedra with aspherical topologies. The customized sequence design was applied to assemble tetrahedron, enhanced hexahedron structures, cubic octahedron, octahedron, and icosahedron. Subsequently, researchers extend the design of transmembrane pores by building larger funnel-like channels based on DNA origami, overcoming the limitations of conventional chemical synthesis in creating large channels.[68] In 2017, researchers used phages to produce ssDNA, which contains a target chain sequence that is interleaved with a self-cleaving "cassette" composed of two zinc-dependent DNAzymes.[69] All the ssDNA required for several types of DNA origamis were generated in shaker flasks, and the feasibility of mass production of a DNA origami nanorod was demonstrated. Subsequently, researchers combined the self-assembly with DNA origami to generate 330 MDa planar rings with a diameter of up to 350 nm, micron-long tubes equivalent in size to certain bacilli, and 3D polyhedral objects (1.2 GDa) with a diameter of up to 450 nm, with assembly yields exceeding 90%.[70] In 2018, researchers presented a computational method for optimizing the binding specificity in symmetrical tile design, based on which a 20-tile object similar to a rhombic triacontahedron was constructed, which extends the dimension of the array from 2D to 3D (Figure 4C).[71] In 2019, researchers created a design tool to algorithmically construct design-specific scaffold sequences and

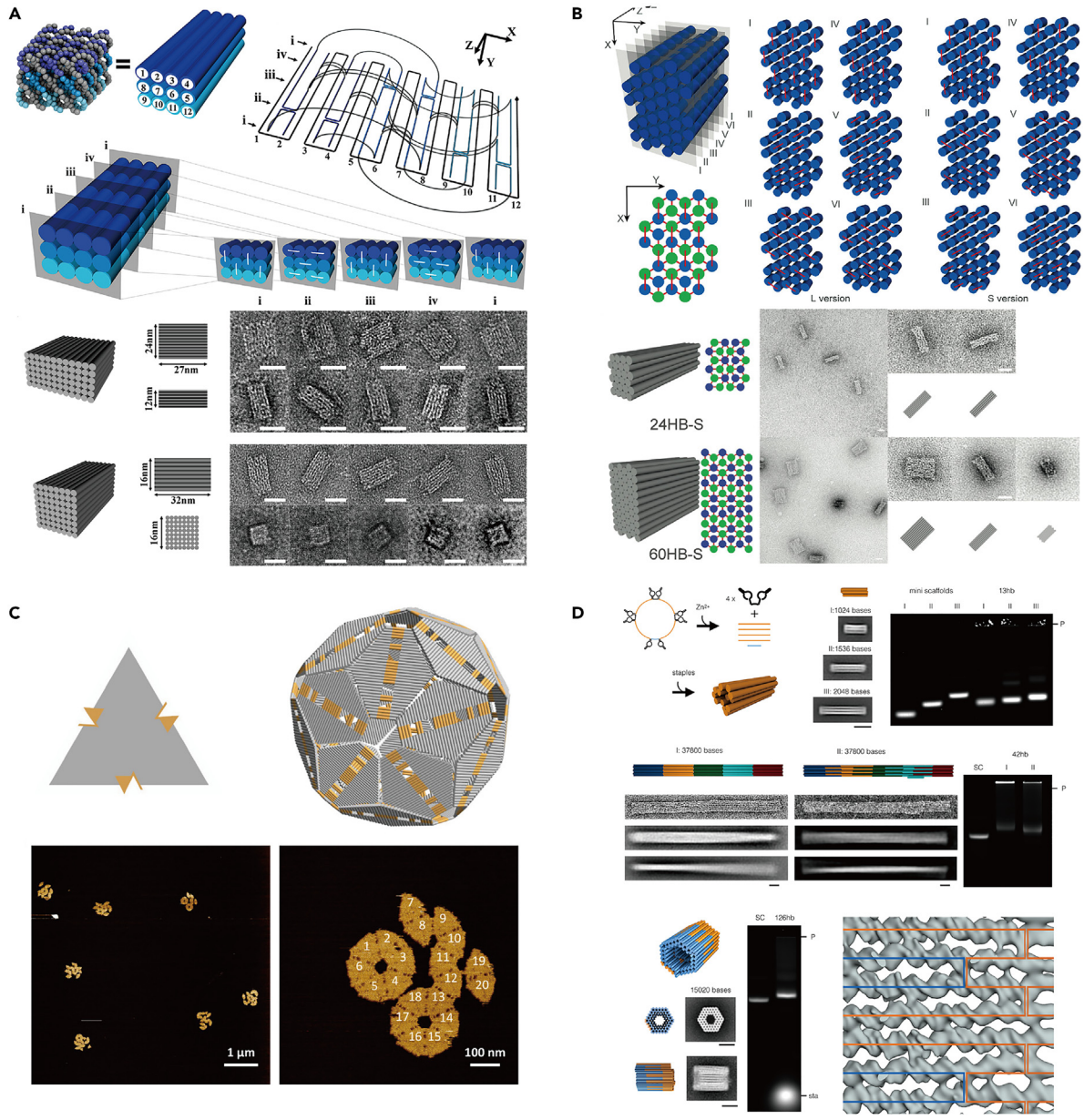

Figure 4. 3D DNA origami structures

(A) Schematic and TEM images of multilayer three-dimensional DNA origamis.[61] Scale bars: 20 nm. Copyright 2009 American Chemical Society.

(B) Model and TEM images of three-dimensional DNA hexagonal-lattice origami.[62] Scale bars: 20 nm. Copyright 2012 American Chemical Society.

(C) Design diagram and AFM image of the polyhedral structure composed of 20 triangular DNA origami tiles.[71] Copyright 2018 American Chemical Society.

(D) Gel electrophoresis analysis and TEM images of different helical bundles.[72] Scale bars: 20 nm. Copyright 2019 American Chemical Society.

presented scalable production approaches to yield scaffold chains with customized sequences (Figure 4D).[72] In this work, a scaffold was built to enable covalent crosslinking of DNA origami upon exposure to ultraviolet irradiation, and a scaffold containing DNAzyme was constructed to realize the separation of the folded DNA origami domain. In 2020, researchers proposed a modular transformation approach by taking advantage of the reconfigurable DNA origami nanoarray, which enables a controllable conversion from 2D structure to 3D architectures.[73] The 2D DNA structure can be modularized into several connected units to enable independent transformation, and such modular conversion can also realize structure conversion between 2D and 3D nanostructures.

APPLICATIONS

DNA origami-based nanofabrication

DNA origami technology is widely used in the manufacture of nanoscale structures (e.g., nanodevices) owing to its capacity to self-fold into well-defined patterns with full addressability that enables manufacture of structures with customized shapes and precise arrangement of heterogeneous elements. A diversity of nanoparticles can be linked to DNA via a series of modification techniques. For example, AuNPs as the representative nanomaterials process unique optical properties and are readily conjugated to thiolated DNA through Au–S bound. Here, we will introduce several representative studies. In 2018, researchers demonstrated the design strategy of chiral DNA nanotubes with customized and addressable inner and outer surfaces.[74] In this study, the external surface can be arranged with chiral distribution of AuNPs to derive a plasma device, while the inner surface can be anchored with the molecular motor orbits enabling cargo transportation within the interior space. In 2019, researchers used the electrostatic interaction between nanomaterials and DNA origamis to produce 3D metal nanoparticle superlattices.[75] Negatively charged six-helix DNA origami and cationic AuNPs were assembled to form a highly ordered 3D tetragonal superlattice. Subsequently, researchers proposed a highly localized metallization reaction based on DNA origami for site-selective metallization patterning with a resolution of 10 nm.[76] Low-valence metal ions (e.g., Cu^{2+} and Ag^{+}) have strong coordination to the bases of protruding clustered DNA (pcDNA) precisely arranged on 2D DNA origami, leading to effective attraction within flexible pcDNA chains for site-selective pcDNA condensation. In addition, mono and double-layered nanoscale-printed circuit board simulant were constructed, opening new horizons for biomimetic manufacturing in the field of nanoelectronics and nanophotonics. Then, researchers presented a general strategy for construction of metal and metal oxide nanoclusters with customized patterns on DNA origami scaffolds (Figure 5A).[77] The main advantage of this method is the addressable arrangement of SH groups on DNA origami that afford active sites to initiate the growth of metal and metal oxide nanoclusters. Researchers demonstrated that DNA origami metamolecules with Fano resonance offers hot spot for single molecules, which enables quantification of the surface-enhanced Raman scattering (SERS) of individual dye molecules.[78] In order to realize customized plasmonic arrangement, a versatile strategy was developed to decorate a set of large AuNPs at the specified docking site of the hyper DNA origami. The tetrameric nanoclusters composed of four spatially arranged large AuNPs (80 nm) present peak-and-dip Fano resonance property. Particularly, the remarkable enhancement at the wavelength of Fano minimum enables the acquisition of SERS spectrum of even an individual signal molecule. In 2020, A DNA origami radiometer was fabricated to measure UV dose by observing the morphological evolution of DNA origami through AFM imaging. Regardless of the morphology (shape and size) of DNA origami, UV exposure could lead to a series of processes from expansion, deformation to eventual disintegration of DNA origami (Figure 5B).[79] Given the positive correlation between the deformation kinetics and the number of nicks in the structure, this UV-driven deformation inspired the generation of a DNA origami-based radiometer to quantify the UV dose in the environment. Subsequently, researchers introduced DNA origami technology into lattice assembly to produce two DNA nanoarchitectures with different symmetries and precisely arrange heterogeneous functional patches on the surfaces with nanometer resolution, providing guidance for further construction of nanoarchitectures.[80] After careful coating with a thin silica layer, these periodic DNA crystals were characterized by electron microscopy and small-angle X-ray scattering. By taking DNA origami as the general temporary template, researchers proposed a three-step approach involving assembly, growth, and lift-off for construction of individual gold nanostructures with well-defined shapes in solution.[81] In this study, AuNPs were precisely immobilized onto the defined binding site of DNA origami scaffold followed by addition of tetrachloroauric (III) acid which functioned as the gold source for *in situ* growth of the AuNP seeds. The surface of the growing seed gradually associated with neighboring AuNP seeds to yield a pre-designed and continuous gold nanostructure, which was finally liberated from the origami scaffold. The templated AuNP seeds with well-defined patterns were successfully converted into corresponding gold structures with the conversion rate of desired structure exceeding 80%. In 2021, researchers proposed a DNA origami-supported metallization strategy in aqueous media to build 3D chiral silver nanostructures (Figure 5C).[82] The diamine silver (I) complex binds to the bases of the single-stranded pcDNA pre-anchored on the DNA origami through coordination, hydrogen bonding, and ion–π interaction. This results in the condensation of pcDNA to locally enrich the silver precursor to facilitate nucleation. A spiral silver pattern with defined chirality and length up to 1 μm was obtained by tubular DNA origami-templated metallization strategy.

In 2015, researchers reported a shape-complementing but non-base pairing strategy to program discrete DNA structures into integrated assemblies.[66] They designed and constructed (i) a reconfigurable gear that

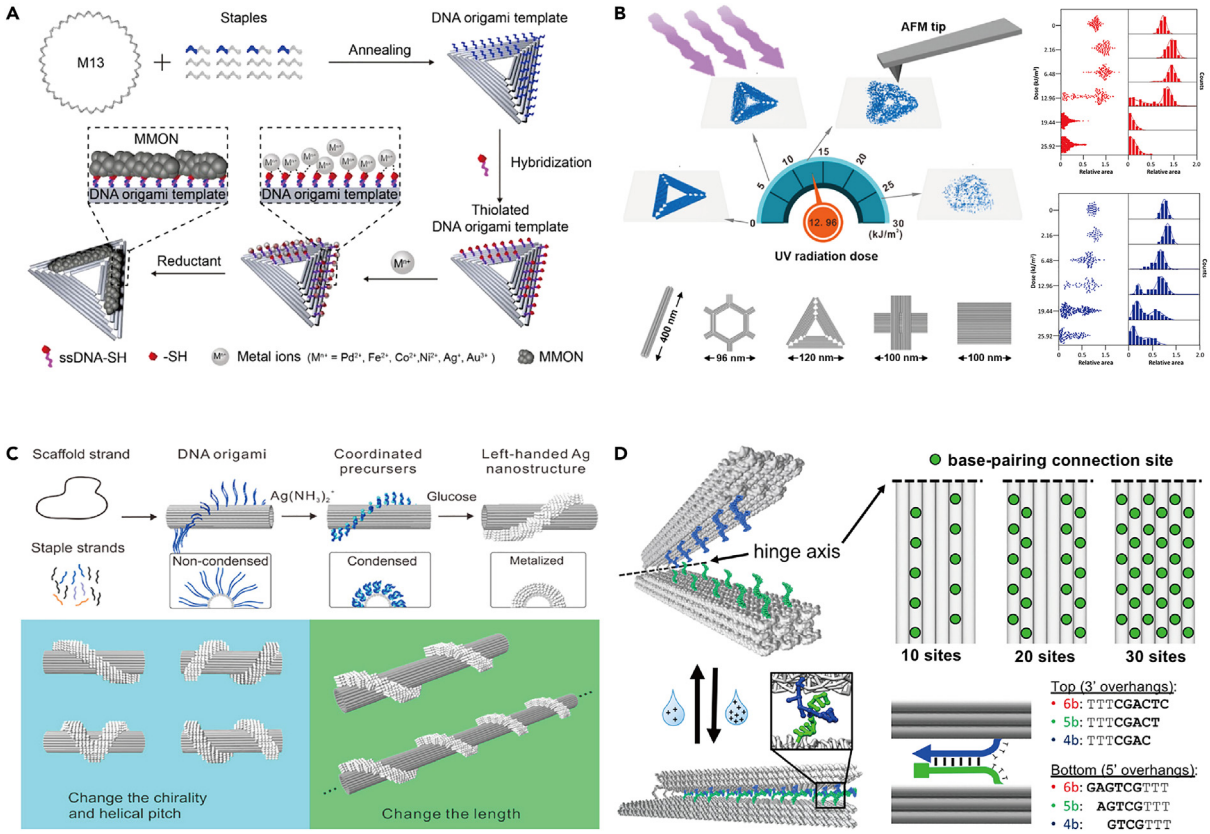

Figure 5. DNA origami-based nanofabrication

(A) Schematic illustrating the site-specific synthesis of metal and metal oxide nanoclusters on a DNA origami template.[77] Copyright 2019 American Chemical Society.

(B) The experimental scheme diagram of DNA origami exposed to different doses of ultraviolet radiation and the statistical distribution of origami area value.[79] Copyright 2020 American Chemical Society.

(C) The assembly procedure of arranging silver patterns with well-defined chirality on a DNA nanotube.[82] Copyright 2021 American Chemical Society.

(D) Ion-triggered reconfiguration of DNA devices.[83] Copyright 2018 American Chemical Society.

can switch between pinion rack-like structure and gear-like shape, (ii) a reconfigurable nanobook capable of reversibly folding and unfolding, and (iii) a heterotrimeric nanorobot composed of three asymmetric subunits that can be reversibly reconfigured into three states namely discrete and organized with open or closed arms. In 2016, researchers assembled large and robust rotaxane objects consisting of DNA origami subunits that can be easily modified to carry small-molecule cargos or even nanomaterials.[84] By combining multi-axis modules, rotaxane structures with shaft length reaching 355 nm were constructed. Particularly, the rotaxane structures were equipped with the fuel/anti-fuel mechanism for manipulating the structural transformation between the fixed and mobile modes. They further assembled pseudorotaxanes, enabling DNA origami rings to slide hundreds of nanometers along supramolecular DNA filaments. Subsequently, researchers demonstrated the orientated assembly of DNA origami onto lithography-defined binding sites to realize controllable coupling of emitters to photonic crystal cavities.[85] By altering the number of growth sites, up to seven DNA origamis were directed to different antinodes inside a cavity, which enables engineering of the cavity emission. In 2018, researchers employed an electrical actuation strategy for sensitive and computer-controlled manipulation of the biocomposite nanorobot system.[86] This electromechanical system is composed of a DNA origami template (55 nm × 55 nm) with an integrated robotic arm (length: 25 nm) that can stretch over 400 nm as driven by an externally imposed electric field. Under the control of computer, precise transformation of the robotic arm between any position on the template can be realized in milliseconds. Subsequently, researchers demonstrated cation-driven conformational change by performing reversible opening and closing of the hinge arm on a sub-second timescale as triggered by different cations (Figure 5D).[83] They further demonstrated the capacity to regulate the responsive behavior which was quantitatively modeled as a function of some design parameters and cation concentration,

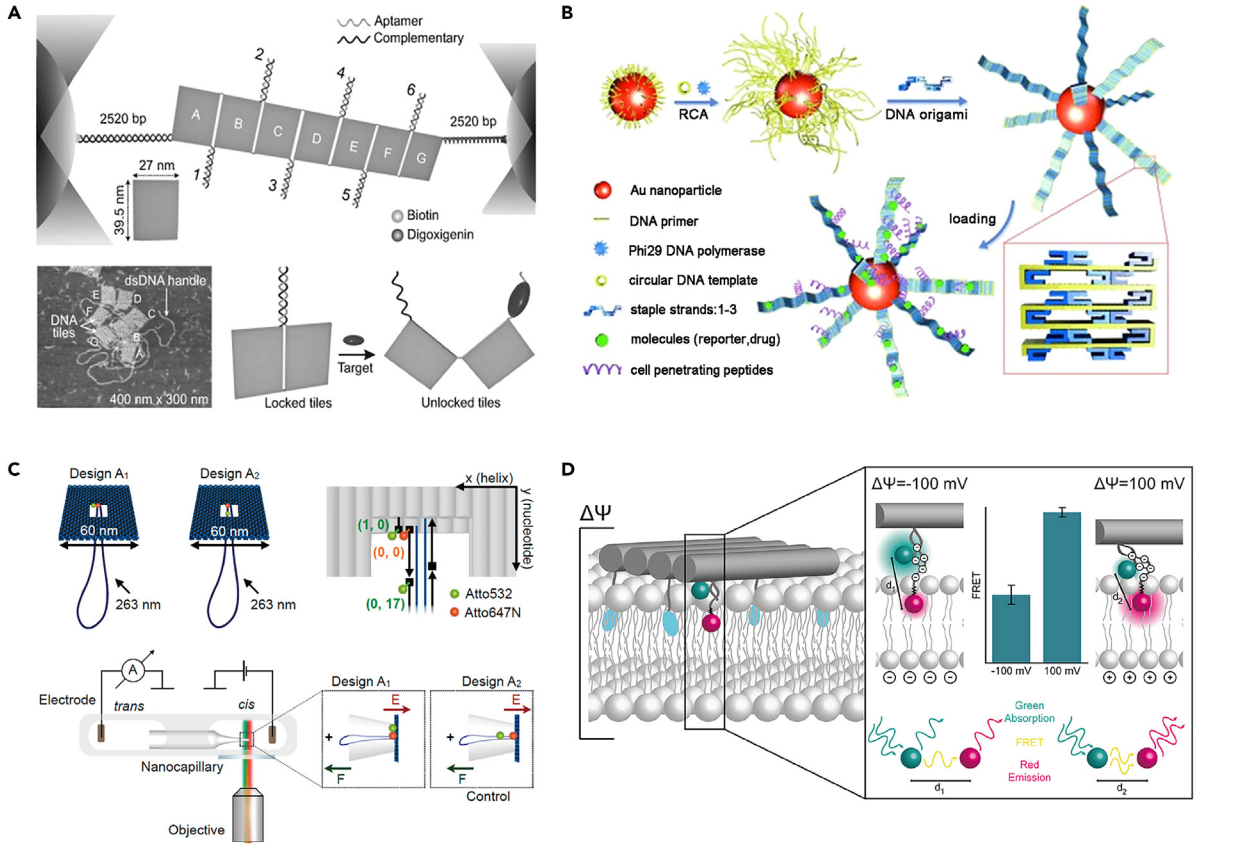

Figure 6. DNA origami-enabled biosensing

(A) Real-time monitoring of DNA origami-based mechanochemical sensing using optical tweezers.[89] Copyright 2014 John Wiley and Sons.

(B) Schematic illustrating the construction of Au-DNA superstructures based on *in-situ* DNA growth and DNA origami folding.[90] Copyright 2015 John Wiley and Sons.

(C) Schematic representation of DNA origami-based optical voltage sensing.[94] Copyright 2018 American Chemical Society.

(D) Schematic illustration of optical voltage sensing based on DNA origami.[96] Copyright 2021 American Chemical Society.

providing guidance for engineering of the range and sensitivity of DNA nanodevice reconstruction. In 2019, researchers presented a imaging and tracking method that realized tracking of DNA rotation at a single-molecule level with fluorescent dye-labeled DNA origami rotors.[87] During RecBCD-mediated DNA unwinding, different stages of initiation, unwinding, pause, and backtracking were monitored, which revealed the initiation mechanism of RecBCD.

DNA origami-enabled biosensing

The nanoscale addressability of DNA origami makes it possible to precisely place diverse sensing elements on complex DNA structures, providing a potential advantage for its applications in biosensing. DNA origami nanostructures offer ultralow detection limits and can be used to monitor dynamic processes directly. In 2011, researchers achieved visual analysis of target nucleotides of interest contained in the probe sequence, in which the effective kinetic methods and AFM technique were conducted to characterize the DNA origami patterns.[88] The graphic corresponding to the origami covers A, T, C, and G nucleotide characters and the symbol containing the test nucleotide disappears when the probe exists. In 2014, researchers developed a new mechanochemical sensing strategy with a 7-block DNA origami nanoassembly using optical tweezers, which provides a unique and universal method for multiplex biosensing (Figure 6A).[89] The combination of multiple recognition sites reduces both the detection limit and detection time. A three-dimensional superstructure comprising a nanoparticle as the core and dozens of two-dimensional DNA structures as the bands was developed (Figure 6B).[90] The DNA band is assembled from long ssDNA grown *in situ* on the surface of the nanoparticle core by rolling circle amplification (RCA). Two

mechanisms are designed to implement molecular payloads on the formed superstructure. One is the merging mechanism, in which ligands bound to the targets are incorporated into DNA structures during the RCA process. The other is the inserting mechanism in which the targets are embedded into the dsDNA generated by DNA origami technique. Particularly, the resultant superstructure in this study integrates the flexibility of DNA structures and the rigidity of nanomaterials, enabling high-efficiency cargo payload for biomedical applications. In 2016, Swati Krishnan et al. introduced a large DNA membrane channel with a pore size of ~4 nm and stable electrical properties, which can be spontaneously inserted into lipid bilayer membranes.[91] Such membrane incorporation can be promoted by either massive hydrophobic modification or streptavidin bridging between biotinylated channels and biotinylated lipids. Consistent with its size, the ohmic conductance of the channel is about 3 ns, allowing the electric-driven translocation of both ssDNA and dsDNA analytes. Confocal microscopy technique was employed to monitor dye influx, which proved that the membrane pores spontaneously formed in the huge monolayer vesicles, and the pores could be oriented in the configuration from outside to inside or inside to outside. Researchers assembled a DNA origami-based nanoscale force clamp that can operate autonomously and enable large-scale parallelization.[92] The ssDNA moieties of the origami functions as entropic springs to exert an adjustable force on the molecular system in the low piconewton range with conformational transition recorded by single-molecule Förster resonance energy transfer. In 2018, Andersen group demonstrated design, assembly, and characterization of a DNA origami-based optical biosensor utilizing a precisely arranged array of fluorescent dyes.[93] In this study, two arrays composed of donor and acceptor fluorophores form a multifluorophore fluorescence resonance energy transfer (FRET) pair, which gives rise to intense signal output for microscopic analysis of a single device. Such single device analysis can realize quantitative analysis of target DNA with concentrations as low as 100 pM in less than half an hour. In the same year, another team designed a voltage-triggered DNA origami, which was rationally labeled with a pair of FRET dye molecules (Figure 6C).[94] The DNA origami was reversibly anchored to the tip of the nanocapillary and underwent tunable structural transformation upon exposure to an electric field which was monitored through variation in FRET efficiency. Particularly, the voltage sensitivity of the DNA origami can be adjusted by altering the position of a single dye, which demonstrates the flexibility and versatility of this method. In 2020, researchers constructed a single-molecule biosensing platform by coupling nanopore readout with recognition system built from DNA origami modified with a targeting aptamer in the central cavity.[95] The modulation of ion current through the nanopore greatly depends on the biomarker bound in the cavity, which allows sensitive biosensing with a detection limit of 3 nM and detection of human C-reactive protein in clinical samples. In 2021, researchers developed an alternative voltage sensor that enables sensing with fluorescent dyes for single-molecule imaging (Figure 6D).[96] In this study, different characteristics (e.g., membrane targeting and voltage sensing modules) were integrated by virtue of DNA origami. A hydrophobic red dye molecule decorated on the lipid membrane and a negatively charged green dye molecule on the DNA origami were utilized to link the membrane dye anchor and DNA structure. The voltage-driven displacement of the anion donor unit was then monitored by the FRET variation of single sensors connected to the lipid membranes.

DNA origami-based drug delivery

The field of nanomedicine benefits from the progress of nanotechnology especially the ability to fabricate nanoobjects as nanovehicles. Among them, DNA nanostructures present great potential in drug delivery due to their full addressability, good biocompatibility, and good control over morphology and surface chemistry. In 2011, researchers developed a CpG oligonucleotide delivery system for endosomal immune activation (Figure 7A).[97] In the case of plain DNA origami tubes and their building units, only non-TLR9-mediated immune response was observed in primary immune cells. The decoration of CpG sequence allows DNA origami tubes to efficiently stimulate the immune activation through TLR9-mediated pathway. In 2012, researchers demonstrated DNA origami as an effective delivery tool to carry anticancer drug doxorubicin (Dox) to inhibit the proliferation of breast cancer cells at a concentration lower than that of free Dox.[98] Rational control of drug release kinetics can be achieved by programming DNA structure with different torsion. In this study, the formulated drug delivery combines good release kinetics, cytotoxicity, and the addressability of the DNA origami to allow prescribed modification of targeting ligands, offering a potent candidate platform for targeted cancer therapy. A smart DNA nanorobot was designed to transport molecular cargo into cells by identifying the inputs from the cell surface to execute logic-gated activation and transforming structure for cargo delivery.[99] This formed device capable of precisely arranging a variety of materials is well controlled by an aptamer-encoded logic gate to realize programmed activation of DNA nanorobot. In 2014, researchers

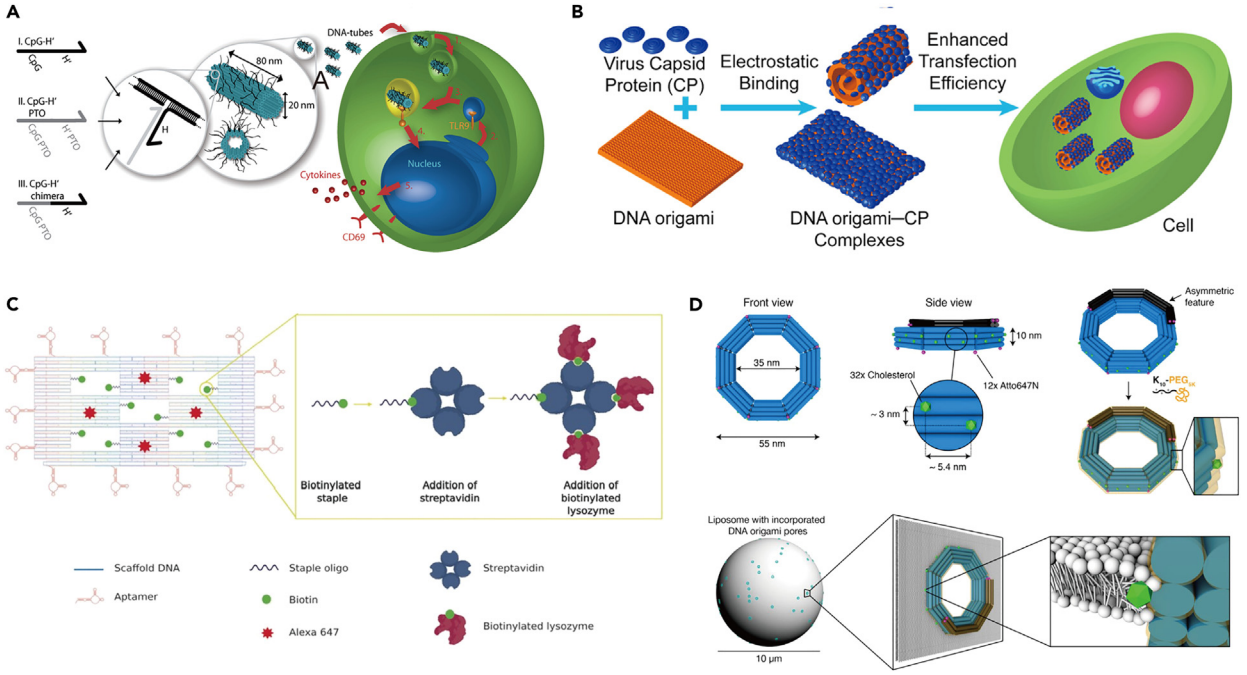

Figure 7. DNA origami for drug delivery

(A) Fabrication of CpG-decorated 30-helix DNA nanotube for cellular immune stimulation.[97] Copyright 2011 American Chemical Society.

(B) Self-assembly of rectangular DNA origami with virus capsid proteins.[100] Copyright 2014 American Chemical Society.

(C) Schematic illustration of DNA origami-based nanocarrier for targeted delivery of antibacterial agents.[104] Copyright 2020 John Wiley and Sons.

(D) Schematic illustrating the design of DNA origami-based ultrawide pore and the insertion method into lipid membrane.[106] Copyright 2021 American Chemical Society.

adopted virus capsid protein to electrostatically decorate on the surface of DNA origami, and further wrapped the origami in the virus capsid to enhance cellular delivery (Figure 7B).[100] Confocal microscopy imaging along with transfection experiments showed that such protein coating presented 13-fold increase of the cellular internalization of DNA origamis compared to the pristine DNA origamis. Subsequently, a biocomputing platform dedicated to manipulating therapeutic reagents in organisms was proposed, which highlighted the potential of DNA origami in fabrication of nanorobots that can dynamically interact with each other *in vivo* to produce logical outputs to switch the molecular cargo on and off.[101] PEGylated liposome can function as an armor layer to prevent the encapsulated DNA nanostructures from nuclease digestion.[102] The grafting of PEGylated liposome also reduces the immune response of DNA nanostructures by two orders of magnitude and increases their bioavailability by 17 times compared with the control group. In 2017, Andersen group reported a DNA origami device capable of packaging enzyme and well manipulating substrate–enzyme interaction through a multi-lock mechanism.[103] Benefiting from the reversible opening/closing under the stimulation of specific key, this DNA nanodevice presented precise control over the enzymatic reaction catalyzed by the embedded protease, showing great potential in diagnosis and drug delivery. In 2020, a bacterial targeting platform combining DNA origami and aptamer technique was developed, which was further modified with antibacterial lysozymes to specifically inhibit bacterial growth (Figure 7C).[104] This DNA origami-based platform features precise targeting and co-delivery of multiple antibacterial agents, affording a powerful tool for fighting antibiotic resistance. In 2021, cellular uptake and penetration in cell globular tissue models (CSTMs) were explored to elucidate whether the internal structure could influence the delivery efficacy of DNA origami.[105] By designing two structures with similar geometry and molecular weight, it is found that the wireframe origamis with lower packaging density and higher local flexibility tend to adhere to the cell surface and penetrate into the deeper region of the CSTMs. In contrast, DNA origamis with tightly packed structure were more likely to be taken up by cells rather than penetrating deeper into CSTMs. Subsequently, researchers incorporated origami pores with ultrawide inner diameter of ~30 nm into the lipid membrane for pore reconstitution, which presents potential in transmembrane transport of macromolecules (Figure 7D).[106]

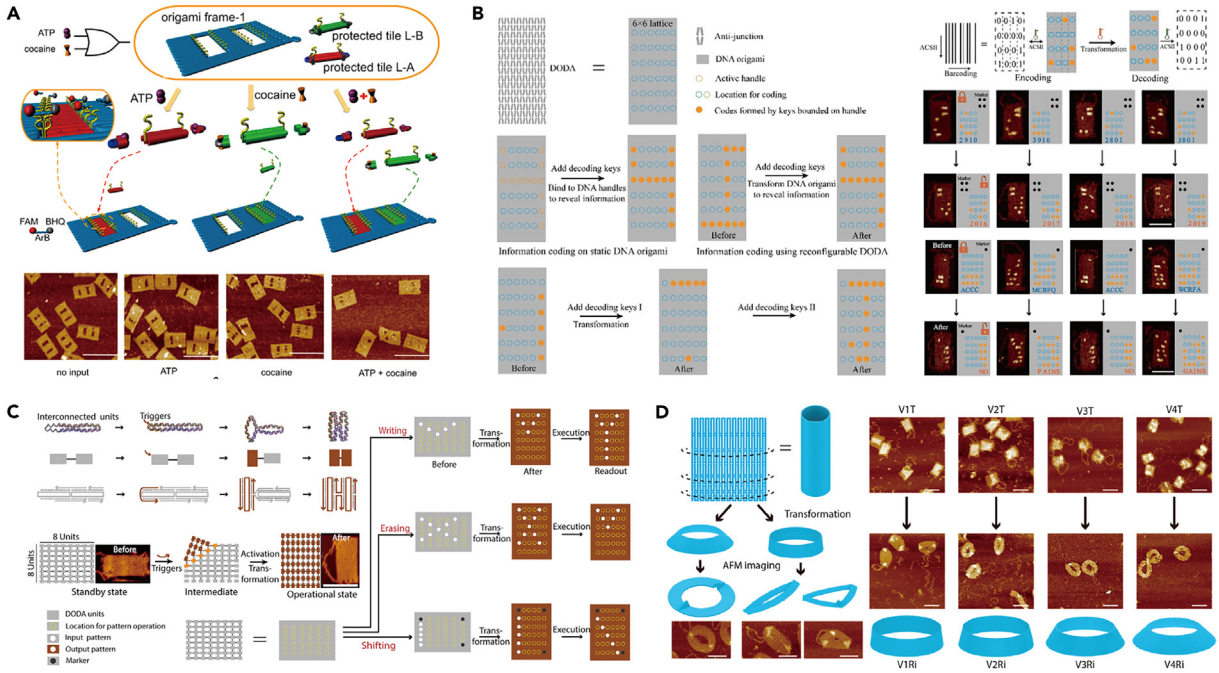

Figure 8. DNA origami-enabled computing and storage

(A) Schematic showing the logical control of DNA tile filling into a preformed DNA origami frame.[112] Scale bars: 200 nm. Copyright 2016 American Chemical Society.

(B) Conformational transition process of reconfigurable DODA and the barcode in DODA. Scale bars: 100 nm. Reprinted from ref. [39] Copyright 2020 John Wiley and Sons.

(C) Reconfigurable DODA-based controllable pattern operations.[38] Scale bars: 100 nm. Copyright 2020 American Chemical Society.

(D) Reconfigurable DNA domino origami-enabled curvature programmability.[117] Scale bars: 100 nm. Copyright 2020 American Chemical Society.

Given that good biostability of nanocarrier is often a prerequisite for efficient drug delivery, some strategies were proposed to strengthen the robustness of DNA origami for biomedical applications. In this context, Auvinen et al. coated DNA origami with a modified BSA layer to enhance the biostability and transfection rate of DNA origami.[107] In this work, dendron was conjugated to BSA to yield one-to-one BSA-dendron hybrid which was further wrapped on the negatively charged DNA origami nanostructure via electrostatic interaction. Subsequently, another team grafted polyethylene glycol–polylysine block copolymer onto different types of DNA origamis via electrostatic interaction, which protected DNA origami against nuclease digestion and low cation concentrations.[108] In 2018, a scalable and site-specific strategy was reported to create additional covalent bonds through UV-triggered cross-linking of proximal thymidines, thereby enhancing the structural stability of DNA origamis.[109] In 2021, Morri group conducted detailed analysis of the staple ligation to optimize the condition for T4 DNA ligase-mediated enzymatic ligation, which elevated the melting point of DNA origami about 5°C–20°C in a structure-dependent manner to enhance the thermo stability of DNA origami.[110] In the same year, self-assembly and reconfigurability of DNA origami were harnessed to repair the defects caused by light or enzyme to improve the stability of DNA origami in complex medium (e.g., fetal bovine serum).[111]

DNA origami-enabled computing and storage

Attributing from the full addressability, DNA origami has emerged as a useful tool for fast computing and efficient storage. In 2016, researchers combined DNA aptamer-substrate recognition and DNAzyme-catalyzed cleavage to control the behavior of DNA tiles filling a pre-designed DNA origami frame by operating a set of DNA logic gates (OR, YES, AND) responsive to adenosine triphosphate and cocaine (Figure 8A).[112] AFM observation as well as fluorescence monitoring over time verified that the DNA origami pattern could be manipulated in a programmable fashion. In 2017, a smart DNA robot was fabricated to operate complex cargo sorting on two-dimensional DNA origami.[113] This DNA origami-based system can realize synchronous sorting of different cargos and multiple robots to cooperatively complete the same task. In 2017, researchers proposed a coding strategy featuring high physical density and virtually unlimited data retrieval

while approaching closer to the Shannon capacity of DNA storage than previously reported designs.[114] It encodes an entire computer operating system, movie, and other files with 2.14×10^6 bytes in DNA and perfectly performs information retrieval from the sequencing range equivalent to an individual tile of Illumina sequencing. In addition, 2.18×10^{15} retrievals can be performed by virtue of DNA samples and the information can be perfectly decoded. In 2018, researchers encoded more than 35 different files with a total size over 200 MB in more than 13 million DNA. In particularly, each file can be correctly recovered via random access.[115] In 2019, researchers proposed DNA origami cryptography (DOC), which assembled the M13 bacteriophage genome DNA into nanoscale braille-like streptavidin patterns for secure message transmission and generates a key of more than 700 bits.[116] Protein binding-based steganography benefiting from the full addressability of DNA origami further enhances the message confidentiality in DOC. In addition, the versatility of DOC is further verified by conveying messages including text, images, and notes. In 2020, researchers demonstrated the feasibility of molecular information coding in a reconfigurable DODA (Figure 8B).[39] A set of keys were applied to transform the encrypted message into a visible form in DODA. In addition, an anti-counterfeiting method based on toehold strand displacement reaction triggered by configurational change is proposed to prevent molecular information coding from decoding and tampering, which provides a highly confidential strategy for message encryption and steganography. After that, the same research group further proposed a reconfigurable DODA-based dynamic mode operation platform. Under the stimulation of trigger strands, the reconfigurable DODA undergoes an autonomous cascade of stacking conformational transition from "before" to "after" pattern, which brings the execution units close enough to trigger strand displacement cascades to complete three distinct operating modes namely writing, erasing, and shifting (Figure 8C).[38] In 2020, researchers reported a dynamic DNA domino origami-based structure capable of switching between straight and curved patterns in the cascade process as activated by trigger strands. Interestingly, the curvature can be well programmed by varying the size of DNA units in DNA origami (Figure 8D).[117]

CHALLENGES AND PERSPECTIVES

DNA nanotechnology has made tremendous progress in the past few decades, among which DNA origami technology has undergone a transition from original design to higher-order two-dimensional and three-dimensional assembly. This review summarizes the advance of DNA origami and its applications in nanofabrication, biosensing, drug delivery, and computational storage.

However, the application of DNA origami is still in its infancy, and its advantages and limitations rely on specific applications. In general, DNA origami technology still faces the following practical challenges: (i) Given the high cost of DNA origami especially DNA staples, it is necessary to develop facile, cost-effective synthesis and assembly strategies to lower the cost yet enhance the yield to favor the practical applications. (ii) Further development of convenient, automated, and efficient design software is urgently demanded to simplify the design process of DNA origami. (iii) The dimension of DNA origami is currently limited due to the lack of diverse DNA scaffolds. To break through this limitation, longer chain DNA scaffolds as a complement to M13 bacteriophage genome DNA,[33,36] high-order assembly,[53] and wireframe DNA origami technology[59] were proposed. Although these strategies are conducive to scaling up the size of DNA origami, more DNA scaffolds or alternative approaches are needed to be developed to fabricate more intricate and larger DNA architectures. (iv) Considering the high dependence of DNA origami on high concentrations of Mg^{2+} and Na^{+} to maintain the structural integrity, and the susceptibility to digestion by nucleases that are ubiquitous in organisms, it is required to develop strategies to strengthen the robustness of DNA origami for biomedical applications and computational storage. At present, some approaches (e.g., covalent linkage of the DNA bases, protein coating, virus-inspired membrane encapsulation, polyethylene glycol grafting, and silica coating) have been explored to enhance the stability of DNA origami.[102,107–109,118,119]

With the improvement of existing technology and the continuous emergence of new strategies, it is expected that DNA origami will play a more critical role and have a broader prospect in their related development and applications.

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AUTHOR CONTRIBUTIONS

Z.H.: Conceptualization and revision; K.S.: Preparation of the original draft and figures; J.L.: Revision; J.C.: Conceptualization, revision, and supervision.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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