



Review Article (Invited)

DNA nanotechnology provides an avenue for the construction of programmable dynamic molecular systems

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Self-assembled supramolecular structures in living cells and their dynamics underlie various cellular events, such as endocytosis, cell migration, intracellular transport, cell metabolism, and gene expression. Spatiotemporally regulated association/dissociation and generation/degradation of assembly components is one of the remarkable features of biological systems. The significant advancement in DNA nanotechnology over the last few decades has enabled the construction of various-shaped nanostructures via programmed self-assembly of sequence-designed oligonucleotides. These nanostructures can further be assembled into micrometer-sized structures, including ordered lattices, tubular structures, macromolecular droplets, and hydrogels. In addition to being a structural material, DNA is adopted to construct artificial molecular

circuits capable of activating/inactivating or producing/decomposing target DNA molecules based on strand displacement or enzymatic reactions. In this review, we provide an overview of recent studies on artificially designed DNA-based self-assembled systems that exhibit dynamic features, such as association/dissociation of components, phase separation, stimulus responsivity, and DNA circuit-regulated structural formation. These biomacromolecule-based, bottom-up approaches for the construction of artificial molecular systems will not only throw light on bio-inspired nano/micro engineering, but also enable us to gain insights into how autonomy and adaptability of living systems can be realized.

Key words: molecular self-assembly, DNA nanotechnology, DNA nanostructures, artificial cell engineering, molecular robotics

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Introduction

Self-assembly is a process by which individual components spontaneously form organized patterns or supramolecular structures. It underlies a variety of cellular

◀ Significance ▶

Self-assembly, which is autonomously and spatiotemporally regulated by dynamic molecular interactions and chemical reaction networks, underlies various essential biological functions, such as cell migration, gene transcription, and cellular metabolism. Creating a molecular self-assembling system resembling a biological system with designed molecules would pave the way for the development of artificial molecular systems. Designability of DNA based on Watson-Crick base pairing has been best employed to construct various-shaped nanostructures and molecular reaction circuits. In this review, we provide an overview of the recent key achievements in DNA nanotechnology that enable us to design and fabricate bio-inspired self-assembled systems.



events. For instance, endocytosis and subsequent vesicle trafficking are often mediated by proteins with self-assembling properties, represented by clathrin, which assembles into a polyhedral lattice on intracellular membranes to form protein-coated vesicles [1]. The basis for cell migration [2] and intracellular transport [3] is provided by self-assembled cytoskeletal filaments, such as actin filaments and microtubules, comprising of actins and tubulins, respectively. Gene transcription [4] and stress granule formation [5] are related to phase separation of proteins and/or nucleic acids, which leads to the formation of molecular condensates. Creating a molecular self-assembling system resembling a biological system will widen our understanding of living cells and accelerate the development of nano/micro technologies inspired by biological systems.

One of the noteworthy features of biological self-assemblies is the dynamic association and dissociation of their assembly components. This property aids in finding appropriate assembly pairs through repetitive attachments and detachments of molecular components, thereby facilitating the formation of correctly assembled structures [6]. Furthermore, it supports the assembly/disassembly of structures in response to environmental changes [7,8]. These implications suggest that designing molecular interactions that allow dynamic association and dissociation is an essential step for the construction of the bio-inspired self-assembly systems using artificially synthesized molecules.

In this review, we provide an overview of the recent studies on artificially designed DNA-based self-assembly systems that exhibit dynamic features, such as regulatable association/dissociation of assembly components, fusion/fission of macromolecular droplets formed by phase separation, environment-dependent assembly, and chemical circuit-regulated structural formation. Following a brief introduction on the general approaches for designing DNA nanostructures and molecular circuits, we focus on the strategies used for realizing dynamic molecular systems that comprise of DNA nanostructures and exhibit responsiveness to changes in the environment, such as salt concentration and solution temperature. We then describe the advances in DNA self-assembled systems coupled with molecular circuits. Finally, we discuss the potential uses of DNA nanotechnology in the construction of artificial cells and molecular robots.

DNA as a programmable material

In addition to its biological role as a carrier of genomic information, DNA is recognized as a programmable material, because of the Watson-Crick base pairing [9], where two complementary DNA segments hybridize into the canonical B-form duplex, a right-handed double-helix

with a diameter of 2 nm and a helical pitch of 3.4 nm [10]. In the field of structural DNA nanotechnology, the programmability derived from the sequence-specific hybridization and the well-defined double helical structure is best employed together with the flexibility of single-stranded DNA for the construction of various-shaped nanostructures from sequence-designed DNA through self-assembly. Oligonucleotides with user-specified sequences are available, because of the significant progress in DNA synthesis technology, which enable designing the inter- and intra-molecular hybridization and their strengths at a single nucleotide level.

A classic example of a basic DNA nanostructure is a DNA junction, such as a three-way and four-way junction (Fig. 1a). A three-way DNA junction is built from three ssDNA components, where each half of each strand hybridizes with a different strand. Similarly, a four-way DNA junction is built from four different strands and often regarded as an 'essence' of DNA nanotechnology, because of its role in connecting two parallel or anti-parallel DNA strands into a DNA bundle. By properly arranging DNA junctions, tetrahedron [11] and cage-shaped [12] DNA nanostructures are constructed on a nanometer scale (Fig. 2a). The range of shapes of DNA nanostructures is widened by the development of sophisticated design approaches, such as DNA tiling [13–15], DNA origami [16–21], and DNA brick [22,23]. Among them, the scaffolded DNA origami method [16] is versatile and routinely used. In this method, long ssDNA (scaffold DNA), typically M13mp18 bacteriophage genome DNA (7249 nucleotides), is folded into a designed shape via hybridization with hundreds of short ssDNA (staple DNA) (Fig. 2b) [16,24]. It allows the fabrication of two-dimensional (2D) or three-dimensional (3D) structures of several tens to a few hundred nanometers (Fig. 2c) [25,26]; these structures are further used as building blocks of higher-order assemblies [27–29].

Besides being a structural material, DNA can be adopted to construct molecular circuits capable of logical computing or exhibiting designated reaction cascades. One of the most typical reactions employed for DNA-based circuit construction is toehold-mediated strand displacement [30,31], which allows for the exchange of a DNA strand in a duplex with a newly added third strand (Fig. 1b). When one of the DNA strands in double-stranded DNA (dsDNA) displays an overhang region (toehold), the DNA strand with the toehold exchanges its hybridization-paired strand with another single-stranded DNA (ssDNA) with a fully complementary sequence. During this reaction, the complementary ssDNA invades the dsDNA through branch migration from the toehold region, because of the thermodynamic stability of the longer duplex, resulting in the exchange of the DNA strand in the duplex. DNA molecules can also be edited using enzymes, such as

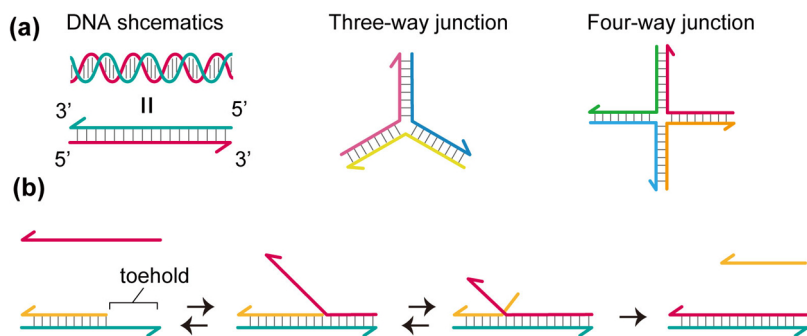


Figure 1 DNA nanostructures and a toehold-mediated strand displacement reaction. (a) Schematic illustration of the double-helix DNA and three- or four-way DNA junctions. (b) Schematic illustration of process of toehold-mediated strand displacement reaction.

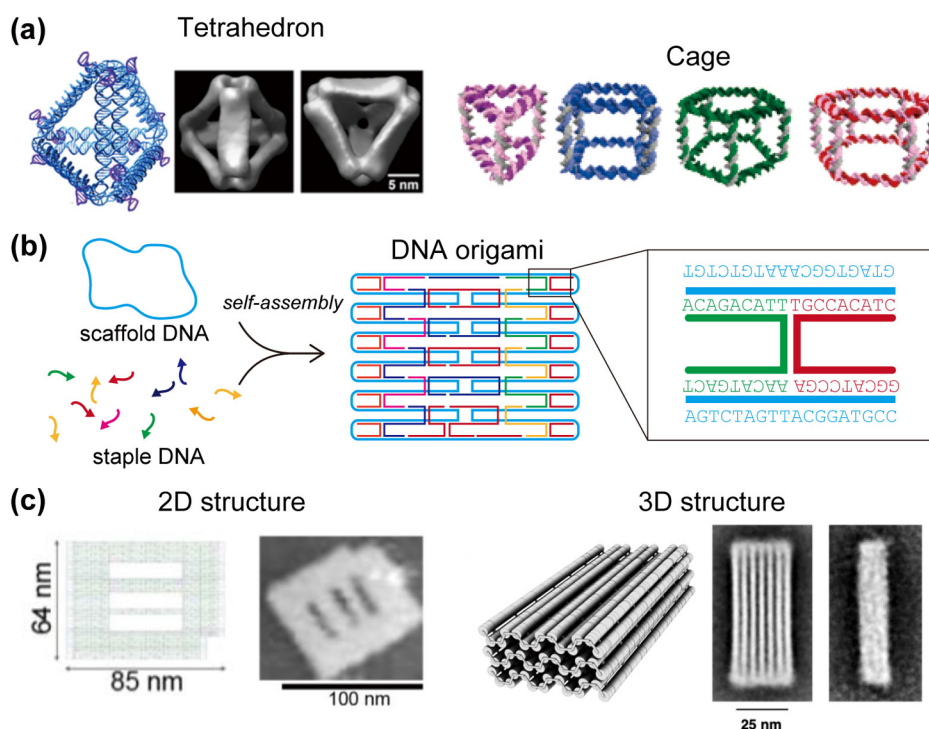


Figure 2 Formation of DNA nanostructures from sequence-designed DNA. (a) Tetrahedron [11] and cage-shaped [12] DNA nanostructures. These images are reproduced with permission [11] and [12]. (b) A schematic representation of the DNA origami nanostructure produced by self-assembly of long (scaffold) and short (staple) single-stranded DNAs. (c) 2D [25] and 3D [26] DNA origami nanostructures. These images are reproduced with permission [25] and [26].

polymerase, nuclease, and ligase, which catalyze extension, digestion, and connection of DNA strands, respectively. Combining these unique features enables the construction of programmable molecular circuits that dynamically produce and/or degrade target nucleic acid molecules in response to input molecules or stimuli [32–38].

Dynamic behavior of DNA nanostructures with sticky ends

Sticky-end hybridization enables specific and stable

binding among DNA nanostructures; and therefore, it is typically used to construct ‘static’ molecular assemblies [39–42]. However, by optimizing the sequences, lengths, and number of sticky ends, it can be employed to construct macromolecular structures that exhibit dynamic behavior in response to the surrounding temperature and/or structural concentration.

Hariadi *et al.* [43] designed rectangular DNA tiles capable of self-assembling into DNA nanotubes [44,45] through sticky-end hybridization (Fig. 3a). The kinetics of the DNA nanotube were investigated via real-time

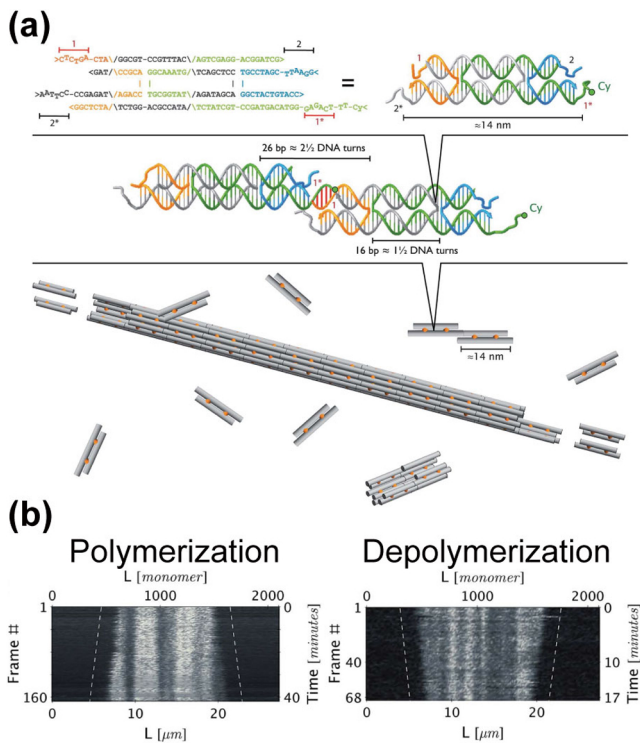


Figure 3 Polymerization and depolymerization of the DNA nanotube [43]. (a) Schematic representation of self-assembly of DNA tiles into a DNA nanotube. The DNA tile was composed of four DNA strands. The tile has 4 6-nucleotide long sticky ends, indicated by 1, 1*, 2, and 2*. (b) Kymographs representing polymerization and depolymerization of DNA nanotubes. The images are reproduced with permission [43].

monitoring of polymerization/depolymerization of the nanotube at the single filament level (Fig. 3b) over a range of temperatures (28.9–41.3°C) and tile concentrations (0–500 nM). The polymerization rate constant of DNA nanotubes ($\sim 6 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$) was comparable to that of actin ($0.5 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ and $7.4 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ for pointed- and barbed-end actins, respectively, at room temperature) [46] and microtubules ($5.4 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$ at 37°C) [47]. The kinetic value will be affected by the sticky end sequences; however, these similarities allowed us to envision the possibilities for the future creation of an artificial cytoskeleton that exhibits treadmilling and/or dynamic instability, which are characteristics of cytoskeletal filaments, such as actin filaments and microtubules.

The dynamic behavior of DNA nanostructures constructed via sticky-end hybridization can also be extended to 3D structures. Sato *et al.* [48] reported the formation of a macromolecular “droplet” via liquid-liquid phase separation (LLPS) of DNA nanostructures. Y-shaped DNA nanostructures (Y-motifs) with three sticky ends were prepared (Fig. 4a). Self-assembly of the Y-motifs into micrometer-sized liquid droplets (DNA droplets) was induced by regulating the temperature of the solution

(Fig. 4b). The DNA droplets exhibited fusion behavior when they collided with one another. This liquid-like property was derived from the dynamic association/dissociation of Y-motifs. Droplet-droplet interaction could be regulated by designing two different Y-motifs, whose sequences were not complementary to each other; thus, selective and exclusive fusion behavior of the DNA droplet was confirmed between the orthogonal motifs (Fig. 4c). Sequence design and enzymatic reaction enabled the induction of DNA droplet fission (Fig. 4d). In recent years, LLPS has attracted considerable attention in the fields of biology and biophysics, since the formation of biological condensates, including droplet- and gel-like structures, inside living cells via LLPS has been implicated in various biological processes [4,5,49,50]; however, the detailed mechanisms of biomolecular LLPS are not yet fully understood. LLPS studies based on DNA nanotechnology [48,51,52] provide a tool to simulate biological phase separation, thereby allowing investigation of the mechanism of macromolecular droplet formation, such as the effects of multivalent interaction [53] or sequence-dependency of nucleic acids on phases separation [54].

Dynamic assembly of DNA nanostructures via base-stacking interactions between blunt-ends of helices

Designing shape-complementarity among the assembly-components is a powerful strategy to realize higher-order assemblies. In living cells, interaction via shape-complementary interfaces is often employed for specific binding of proteins to their respective targets. For example, a pocket of ribonuclease P binds to pre-transfer RNA based on shape complementarity [55]. Inspired by this phenomenon, Gerling *et al.* [56] designed 3D DNA origami blocks with shape-complementary recession-protrusion patterns with blunt ends (Fig. 5a). DNA is a negatively charged polymer molecule; and therefore, there is an electrostatic repulsion between the DNA helices; however, this repulsion can be weakened by increasing the cation concentration, enabling interaction between the shape-complementary interfaces, which are further stabilized by base-stacking interactions between the blunt-ends of the helices. Based on this mechanism, cuboidal DNA origami components with self-complementary protrusions and recessions were assembled into a filament-like structure in the presence of 25 mM MgCl_2 . The filament formation was reversible. On decreasing the MgCl_2 concentration from 25 mM to 5 mM, the filament disassembled into monomers. On increasing the MgCl_2 concentration back to 25 mM, the monomers self-assembled into filaments again (Fig. 5b).

Self-assembly coupled with dynamic shape reconfiguration of the components is achieved by introducing structural flexibility into the DNA origami nanostructure.

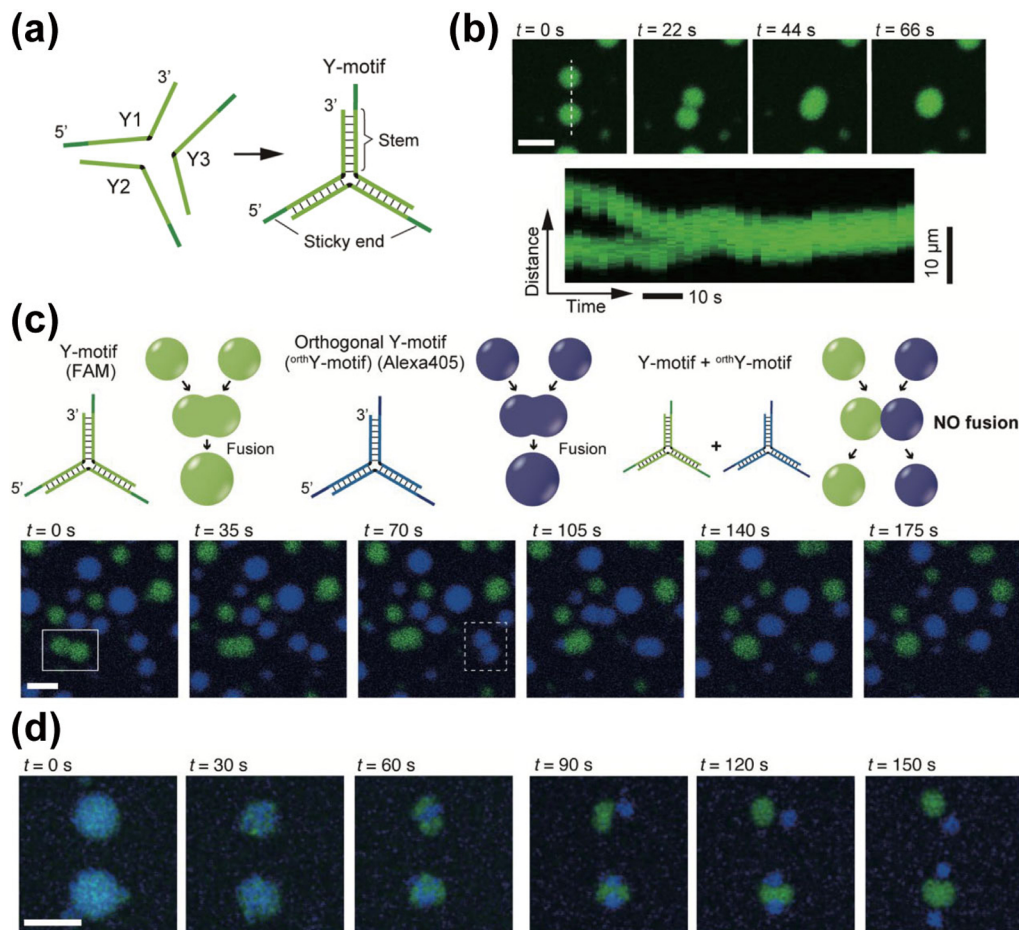


Figure 4 Formation of DNA droplets via dynamic association/dissociation of DNA nanostructures [48]. (a) Schematic representation of the Y-shaped DNA nanostructure (Y-motif) with three sticky ends. (b) Sequential micrographs and a kymograph on the white dashed line in the micrographs represent fusion of the DNA droplets formed by liquid-liquid phase separation (LLPS) of Y-motifs. Scale bar, 10 μm . (c) Sequential micrographs represent selective and exclusive fusion of the DNA droplets. Two types of droplets were formed by Y-motif and orthogonal Y-motif that possesses orthogonal sequences to the Y-motif. Boxes with solid and dashed lines in the micrographs indicate fusion of the droplets with Y-motifs and orthogonal Y-motifs, respectively. Scale bar, 10 μm . (d) Sequential micrographs represent fission of the DNA droplets. Scale bar, 20 μm . The images are reproduced with permission [48].

Cervantes-Salguero *et al.* [57] fabricated shape-reconfigurable DNA origami with a flexible hinge, blunt-ended bonding edges, and springs that limit the movable angle of the hinge (Fig. 6a). The flexible shape of this DNA origami enabled self-assembly into various closed multimers (Fig. 6b). When the DNA origami components were deposited onto a mica substrate with NaCl, surface diffusion and self-assembly into multimers through dynamic shape-reconfiguration were observed (Fig. 6c). Moreover, 3 DNA origami components initially formed an open 3-mer and then reconfigured to form a closed 3-mer. Similarly, two 2-mers self-assembled into a 4-mer via shape-reconfiguration of each monomer. These findings demonstrated the potential use of shape-reconfigurable DNA origami in constructing variously shaped assemblies from a single type of component.

In addition to solid surfaces, such as glass and mica,

soft surfaces have also attracted attention as supporting structures for 2D self-assembly. Suzuki *et al.* [58] reported the lipid-bilayer-supported self-assembly of 2D DNA origami lattices (Fig. 7a). In their study, cross-shaped DNA origami structures with blunt ends were electrostatically adsorbed onto a mica-supported ‘fluidic’ lipid bilayer in the buffer containing 10 mM MgCl_2 and 1 mM EDTA. The surface-adsorbed origami structures exhibited 2D diffusion and self-assembly into micrometer-sized lattices (Fig. 7b), accompanying dynamic processes, such as lattice fusion, boundary reorganization, and defect filling (Fig. 7c). It should be noted that 2D lattice formation depends on the lipid phase; however, it can be tuned by ionic conditions. Indeed, under the same buffer conditions, the origami structures were less mobile and packed into aggregates on the ‘solid’ lipid bilayer. However, these disordered aggregates were reorganized into ordered lattices through

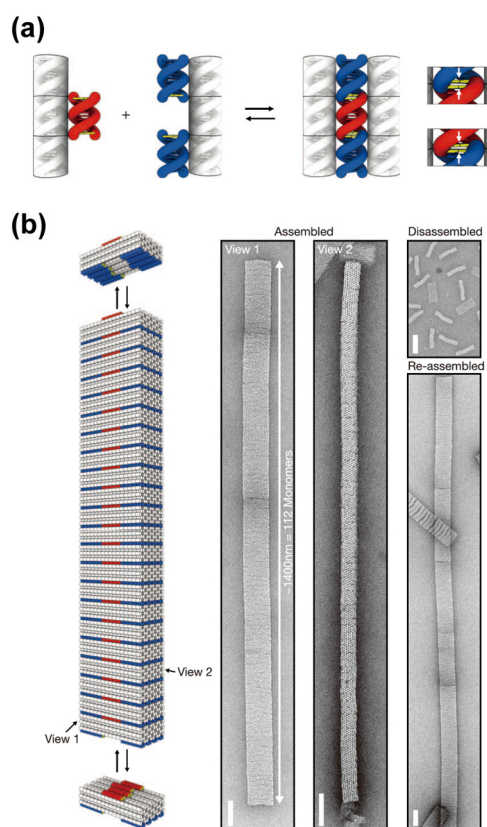


Figure 5 Reversible self-assembly of DNA origami components via shape-complementarity and blunt-end stacking [56]. (a) Schematic representation of blunt-end stacking interaction between shape-complementary DNA nanostructures. The protrusion and recession parts of the DNA helix are represented by red and blue colors, respectively. White arrows in right insets represent π - π stacking interaction. (b) Schematic and negative-stain transmission electron micrographs of shape-complementary blunt-end stacking-based reversible multimerization of DNA origami components. Filament-like multimers were assembled in the presence of 25 mM MgCl_2 . The filament was disassembled in the presence of 5 mM MgCl_2 and re-assembled by increasing the cation concentration to 25 mM MgCl_2 . Scale bar, 50 nm. The images are reproduced with permission [56].

weakening of the interaction between DNA and the bilayer surface via NaCl addition [59]. These dynamic properties of DNA origami assemblies may provide clues on the construction of molecular systems capable of self-healing and self-adapting to specific environmental conditions.

Self-assembly regulated by a DNA-based molecular circuit

A molecular circuit made of synthetic DNA and enzymes offers a method to control self-assembly of DNA nanostructures. Green *et al.* [60] achieved autonomous control of DNA nanotube assembly using a synthetic molecular circuit. The DNA nanotube was assembled using DNA tiles with toehold domains. The toehold-mediated strand

displacement reaction with invader strands induced the disassembly of DNA nanotubes. The authors used an oscillator circuit [30], in which RNA invader strands were produced by RNA polymerase and degraded by ribonuclease H. By coupling the circuit to the nanotube system, repetitive assembly/disassembly of the nanotube was successfully achieved.

Hamada *et al.* [61] developed a more complex system to demonstrate artificial metabolism-like reactions (sequential production and decomposition of materials) using DNA and enzymes in a microfluidic device (Fig. 8a). Long ssDNA was synthesized in a flow-containing mixture via a rolling circular amplification reaction [62], resulting in the formation of a mesoscale DNA-based Assembly and Synthesis of Hierarchical (DASH) pattern in the device (Fig. 8b). The DASH pattern was generated by DNA hydrogel networks (entanglements of long ssDNA); therefore, it could be decomposed by DNase. The production/decomposition mechanism was explained as an autonomous state transition among 'Init-,' 'Growth-,' and 'Decay-' states (Fig. 7c). Initially, materials were supplied to the device (Init-state). Then, DNA hydrogels were formed between the pillars (Growth-state). The formation of hydrogel networks altered the flow dynamics, resulting in the initiation of the degradation process (Decay-state). The chemical reaction pathways and sophisticated device design successfully and autonomously achieved the sequential occurrence of generation and degradation (Fig. 8c). This study suggested that artificial molecular reaction circuits have the potential to realize a transient molecular assembly/disassembly and production/decomposition, which is an essential feature to bring cells to life.

Conclusion and outlook

DNA nanotechnology has enabled the construction of various-shaped nanostructures capable of self-assembling into supramolecular structures. It has also promoted the development of artificial molecular circuits that produce/degrade DNA with a specific sequence. Reversible supramolecular assembly, including re-organization, dynamic structural formation/decomposition, and liquid droplet formation via LLPS of DNA nanostructures, was achieved by designing shape complementarity, adjusting interaction strengths, and regulating the solution temperature. Autonomous molecular self-assembly was realized using an artificial molecular circuit with sequence-designed DNA and enzymes. Although theoretical approaches based on free energy of association and dissociation of assembly components are still desired to be studied, these experimental achievements demonstrate the potential use of DNA nanotechnology in realizing artificial molecular systems that are reminiscent of biological

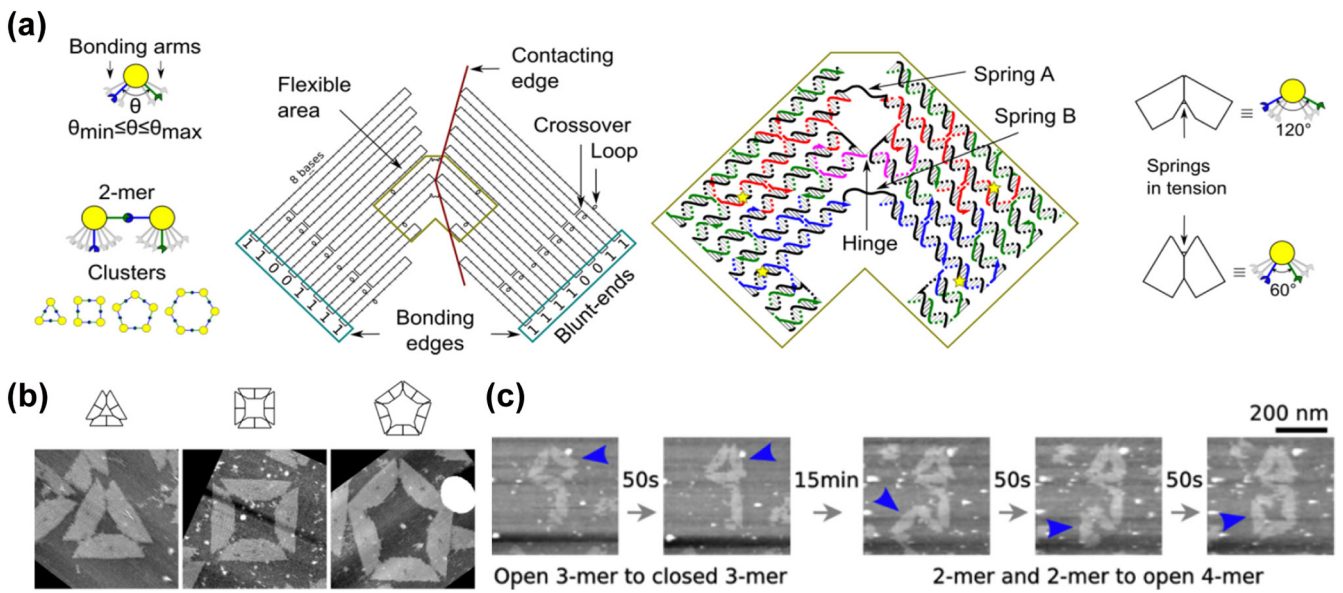


Figure 6 Self-assembly of shape-reconfigurable DNA origami components via blunt-end stacking [57]. (a) Concept and schematic representation of shape-reconfigurable DNA origami with a hinge, bonding edges, and springs that determine the angle of the bonding arm. (b) Atomic force microscopy (AFM) images (310×300 nm) of 3-mers, 4-mers, and 5-mers that comprised shape-reconfigurable origami structures. (c) Sequential AFM images representing the dynamic process of self-assembly of shape-reconfigurable DNA origami components. The images are reproduced with permission [57].

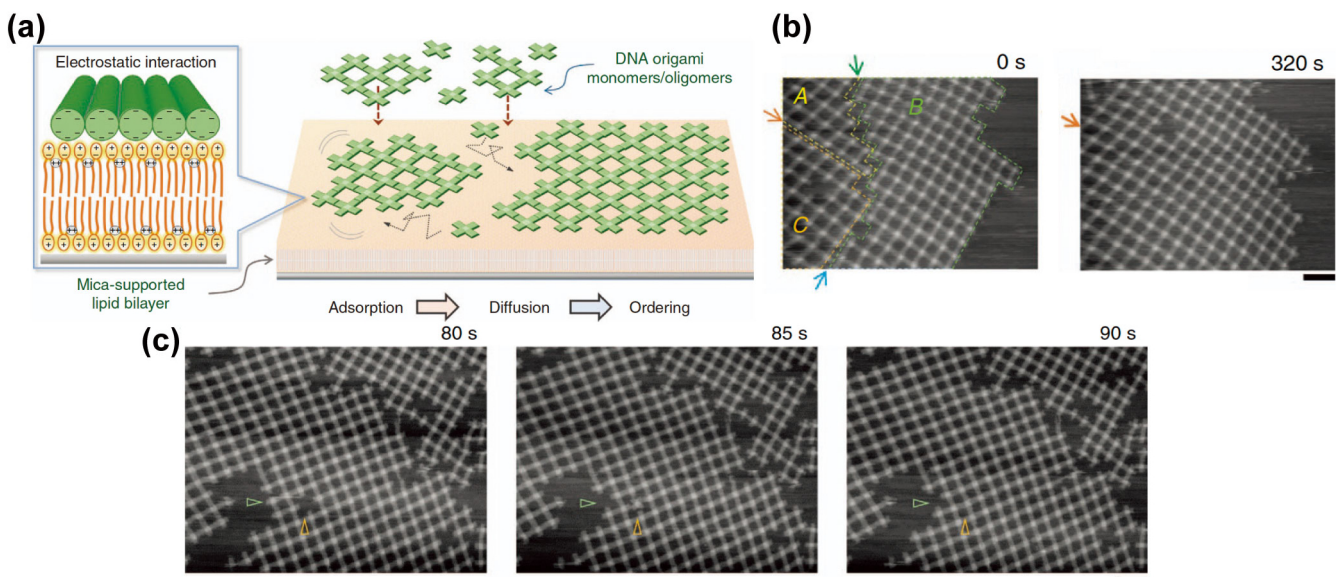


Figure 7 Self-assembly of DNA origami components on the lipid bilayer membrane [58]. (a) Schematic representation of the interaction between DNA origami components and mica-supported lipid bilayer (SLB) and lattice formation of cross-shaped DNA origami components on the SLB. The interaction between the DNA origami components and SLB was mediated by divalent cations. Since the ends of each edge of the DNA origami were blunt, the DNA origami components could self-assemble into lattices via stacking interaction. (b) Reorganization of multiple DNA origami lattices into a single large lattice. Three lattices (indicated by A, B, and C) were fused by repetitive association and dissociation. (c) Defect filling observed in the DNA origami lattice. Point defect in the lattice was filled with the monomer. Scale bar, 200 nm. The images are reproduced with permission [58].

systems mainly composed of protein molecules.

In biological systems, information encoded in the genomic DNA is transcribed into an RNA molecule and

then translated into a polypeptide chain, which is further folded into a functional 3D structure, the protein. In DNA-based artificial systems, although they are similar to

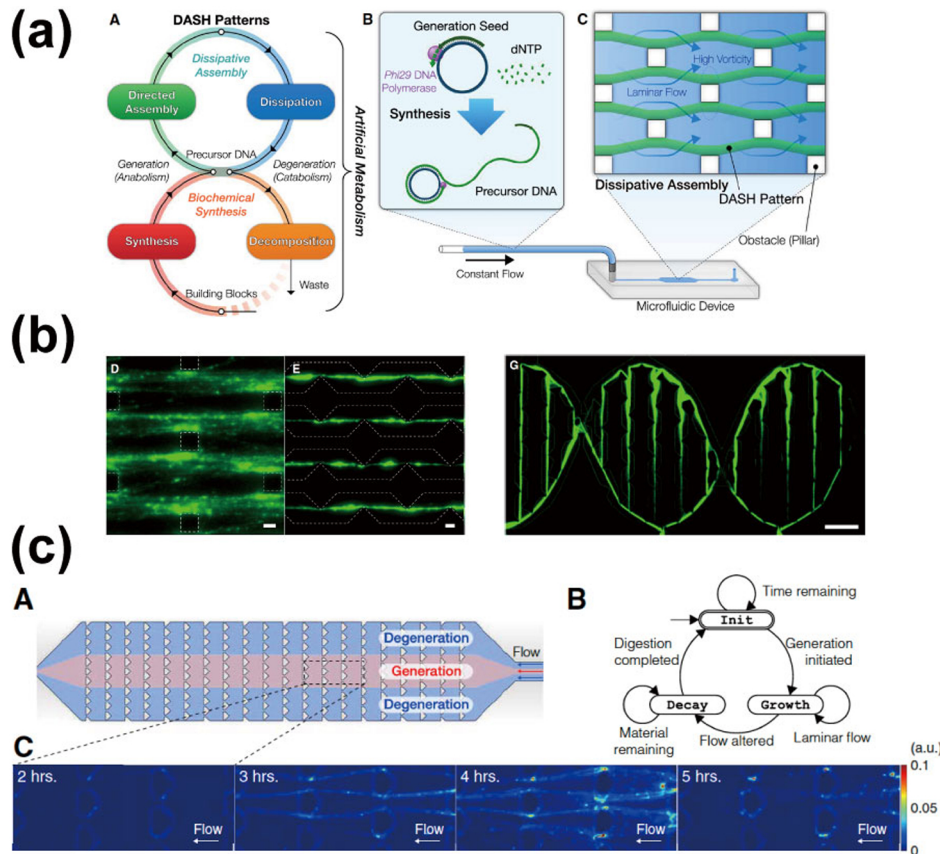


Figure 8 Artificial metabolism-like reaction achieved using DNA, enzymes, and a microfluidic device [61]. (a) Schematic illustrations of the mechanism of DNA-based Assembly and Synthesis of Hierarchical (DASH) materials. DNA hydrogels were produced as the DASH pattern by rolling circular amplification in the microfluidic device. (b) Generated mesoscale DASH patterns. Scale bars, 10 μm (left panel) and 50 μm (right panel). (c) Sequential generation and degeneration of DASH patterns via artificial metabolism-like reaction. The images are reproduced with permission [61].

biological systems in that DNA sequences provide blueprint of the systems, DNA molecules themselves are folded into designed structures and exhibit prescribed functions. Functions realized by DNA nanotechnology is not limited to those achieved by natural nucleotides. Artificial nucleic acids and nucleotides with chemical modifications provide means to expand the functionality of DNA nanostructures.

One of the limitations in DNA-based artificial systems is that they cannot spontaneously initiate optimization, while the natural systems are optimized through evolution and natural selection. The recent success in machine-learning-based improvement of protein functions [63], using *in silico* approaches, offer powerful means to optimize structural components and reaction networks of artificial systems to enhance their functions.

Combining bottom-up molecular designing and top-down micro-device engineering may also provide an alternative approach to realize more sophisticated systems. As described in this review, metabolism-like sequential production and decomposition of structural materials was

demonstrated using the DNA-based reaction network with the aid of the microfluidic device. Considering that microfluidic devices are often used to model non-equilibrium cellular systems, such as those of oscillatory gene expression [64] and molecular transportation [65], the combination of microfluidics and DNA nanotechnology may lead to the construction of a dynamic artificial molecular system based on energy dissipation.

It should be noted that various techniques, such as induction of changes in ionic strength [7] and/or pH [8], light-irradiation [66,67], and addition of fuel molecules [68,69], can be employed to trigger self-assembly of DNA nanostructures, enabling the construction of various artificial signal-responsive molecular systems that exhibit deformation [67], locomotion [70], swarm behaviors [71], and production of components of supramolecular structures [72]. The recent development of reversible mechanical motion of DNA nanostructures [73] and DNA-based circuits [74] with modularity would empower DNA nanotechnology to enable designing a molecular system that possesses adaptability to environmental changes

[75,76]. We anticipate that a bottom-up approach towards the construction of these artificial molecular systems, such as artificial cells and molecular robots [77–80], will elucidate how autonomy and adaptability of living systems can be realized.

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Conflicts of Interest

The authors declare no competing financial interests.

Author Contributions

Y.Sa. and Y.Su wrote the manuscript. Y.Sa prepared the figures.

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