Dynamic regulation of DNA nanostructures by noncanonical nucleic acids

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Abstract

DNA nanostructures are among the most fascinating self-assembled nanostructures in diverse areas of science and technology, because of their nanoscale precision in biomolecule and nanoparticle organization. The implementation of dynamic and spatial regulation in structural morphology and hierarchical assembly upon specific external stimuli will greatly expand their applications in biocomputation, clinical diagnosis, and cancer therapy. Recently, noncanonical nucleic acids, particularly DNA triplexes, i-motifs, and G-quadruplexes, have become powerful tools for biosensing and mechanical switching. Developments in incorporating stimuli-responsive noncanonical nucleic acids into DNA nanostructures provide a promising approach to regulating the spatial organization and hierarchical assembly of DNA nanostructures. In this review, we briefly introduce recent progress in constructing DNA nanostructures with dynamic regulation of the structural transformation and programmable assembly pathways at the nanometer scale by noncanonical nucleic acids and discuss their potential applications and challenges.

Introduction

Since Nardaran Seeman first constructed artificial DNA nanostructures in the 1980s, well-defined nanoscale DNA architectures have been designed and fabricated, including cubes, tetrahedrons, polyhedra, DNA origami, and spherical nucleic acids (SNAs) (Fig. 1a). Owing to their nanoscale precision in organizing and manipulating molecules, these DNA nanostructures have attracted intense and widespread interest in cutting-edge research areas of physiological studies, clinical diagnosis, cancer therapy, and biocomputing. Currently, developments in harnessing the structural and material properties of DNA nanostructures, including their size at different scales, topologic shape, and spatial arrangement, have been achieved. The next major challenge in DNA nanostructure functionalization is the dynamic regulation of their structural transformation and hierarchical assembly. In particular, reconfigurable and dynamic DNA nanostructures, which can be subjected to on-demand regulation of the morphology, motion and, material properties in response to various external stimuli, play a critical role in a spectrum of fields, including nanorobotics, nanophotonics, nanosensors, nanoelectronics, and nanomedicine. To this end, many efforts have been made in recent years to develop bottom-up methods for functionalizing DNA nanostructures to carry out their structural reconfiguration or manipulate assembly behavior with programmed tunability, revertability and specificity in molecular recognition.

In addition to the right-handed B-form double helix, a variety of nucleic acid conformations that disobey the Watson–Crick canon, particularly the DNA triplex, G-quadruplex, and i-motif (Fig. 1b), are highly attractive functional modules in constructing dynamic and sophisticated DNA nanostructures. Specifically, the DNA triplex is self-assembled by a DNA duplex hybridizing with a third ssDNA through either Hoogsteen or reverse Hoogsteen hydrogen bonding, with preferred base triplets including...
G-quadruplexes are formed by alkali-metal-assisted stacking of guanine (G) tetrads held by Hoogsteen base pairs, while i-motifs adopt cytosine (C)–cytosinium (CH⁺) base pairs resulting from the protonation of N-3 on cytosine. The difference in their structural formation requirements from their duplex counterparts brings about novel capacity in manipulating DNA nanostructures in many ways that would not have been possible by using solely DNA duplexes.

Taking advantage of DNA nanotechnology, researchers have successfully designed and assembled a series of noncanonical nucleic acid-incorporated DNA nanostructures with dynamic regulation in structural transformation and hierarchical assembly (Fig. 1c and Table 1). For instance, by embedding noncanonical nucleic acids into the structures of individual DNA constructs, the distance between certain components can be adjusted, thereby altering the morphology or configuration of the DNA constructs. Moreover, reversible assembly/disassembly of the DNA nanostructures, as well as their spatial rearrangement, can be achieved. In this review, we focus on new progress in utilizing noncanonical nucleic acids to dynamically regulate the structural reconfiguration and hierarchical assembly of artificial DNA nanostructures, which hold wider implications for expanding and regulating the functionalities of materials beyond nucleic acids. Furthermore, we discuss the respective roles and interplay of artificial DNA nanostructures and naturally occurring noncanonical nucleic acids in various applications, including nanosensors, nanomedicine and, nanophononics.

Reconfigurable elements for regulating DNA nanostructures

Noncanonical nucleic acid conformations have been characterized in vitro with various methods, such as fiber
diffraction, NMR spectra, and X-ray crystallography, and have been visualized recently in human cells. In addition to executing regulatory functions in various cellular processes, including DNA replication, transcription, and recombination, they have been widely utilized in the DNA nanotechnology field as reconfigurable elements, owing to their peculiar conformations, properties, and functionalities.

First, noncanonical nucleic acids exhibit a high degree of conformational polymorphism, enabling the fabrication of DNA nanostructures with outstanding structural complexity and diversity. For instance, the strand molecularity of noncanonical nucleic acids varies from 1 to 4. Both triplex and tetraplex nucleic acids can be formed by the assembly of one, two, three, or four oligonucleotide strands, existing as either intra- or intermolecular conformations. The commonly applied conformational switching strategies are summarized in Fig. 2: (a) Intramolecular conformational switching of monomeric triplex/tetraplex DNA. (b) Intermolecular conformational switching of a dimeric triplex/tetraplex. In this strategy, short duplex or hairpin DNA is incorporated into the intertriplex/intertetraplex conformation to enhance its thermodynamic stability and to prevent the formation of undesired structures. (c) Switching between intramolecular and duplexed conformations by introducing a strand with partial complementarity to the triplex/tetraplex-forming strand. This strategy possesses inherent hybridization, directionality and benefits, and the dictation of programmable assembly.

Moreover, the thermodynamic stabilities of noncanonical nucleic acids are determined by their sequence compositions and lengths. This feature provides immense room for the rational design of noncanonical nucleic acid-based functional modules with on-demand performance in hybridization. The formation of the G-quadruplex or i-motif requires four stretches of at least two consecutive Gs or Cs. Stretches of six or more G- or C-residues are long enough to fold back on themselves, making it possible for them to fold into either of the two conformations (i.e., intra- and intermolecular) in a tetraplex structure. For instance, oligonucleotide C₆T₃C₆ folds into an intermolecular tetraplex structure, while C₆T₃C₆, C₇T₃C₇, and C₇T₃C₇-fold into intramolecular conformations. Thus, sequences consisting of 3–5 consecutive Gs or Cs are commonly used in DNA nanotechnology. Triplexes can form only with at least five TAT base triplets in their stems at room temperature, with longer stems giving generally higher stability. Noncanonical nucleic acids can also accommodate a range of folding topologies. G-quadruplexes manifest at best topological complexity: intramolecular DNA G-quadruplexes can theoretically adopt 26 folding topologies, classified based on a number of interdependent structural parameters, including the glycosidic bond configuration, groove width, and loop position. In terms of the relative orientations for adjacent strands, either two, three or all four strands in a G-quadruplex can be oriented in the same direction, while the rest, in the opposite direction. For dimeric i-motif DNA, the length of C-tracts determines the topology to be either head-to-head or head-to-tail (classified based on the relative position of the two loops). Once the C-tracts contain an unequal

| Noncanonical nucleic acid | DNA nanostructure | Functionality | Application | Ref. |
|---------------------------|-------------------|---------------|-------------|-----|
| Triplex                   | 2D origami        | Structural isomerization depending on pH | Programmed assembly | 32  |
|                           | 3D origami        | Opening/closing of origami capsule depending on pH | Drug delivery | 64  |
|                           | SNA               | Releasing drug-loaded SNA in acidic tumor microenvironment | Drug delivery | 70  |
| i-Motif                   | DNA tetrahedron   | Cavity volume regulation depending on pH | Nano-pump | 52  |
|                           | DNA triangular prism | Opening/closing of DNA cage depending on pH | Catalysis | 63  |
|                           | DNA triangular prism | Dimerization depending on pH and ATP | Logic gate/ATP imaging | 31  |
| G-quadruplex              | DNA triangle      | Dimerization depending on K⁺ | Catalysis | 30  |
|                           | 2D origami        | Intertriplex distance alteration by UV irradiation | Optical reporter | 38  |
|                           | 3D origami        | Structural rearrangement depending on K⁺ | Catalysis | 33  |
|                           | 3D origami        | Curvature change depending on K⁺ | Nano-motor | 40  |

Table 1 Overview of DNA nanostructures regulated with noncanonical nucleic acids.
number of C-residues, only the head-to-head arrangement is thermodynamically favored. In triplexes, the third strand can bind to the duplex motif in either parallel or antiparallel orientation with respect to the central strand, with the parallel topology exhibiting higher thermal stability and less tendency to form mismatches.

In addition, given that the formation of noncanonical nucleic acids depends on external stimuli, including pH, temperature, ionic strength, and noble metals, this stimuliresponsiveness greatly enriches the regulatory mechanism of the ever-developing DNA nanotechnology field. For example, the applicable pH ranges of the triplex and i-motif are pH 4.5–10 and pH 5–7.4, respectively. They are able to fold at salt concentrations ranging from physiological to high, nonphysiological levels (e.g., 10 mM MgCl₂ or 315 mM KCl). G-quadruplexes are stabilized optimally by potassium ions (K⁺), and the applicable K⁺ concentration range is ~0.1–1000 mM. Notably, because these environmentally friendly stimuli can be readily implemented in the laboratory to induce DNA conformational switching, the three DNA conformations (tripllex, i-motif, and G-quadruplex) have been more frequently adopted for functionalizing or regulating DNA nanostructures than other non-B DNA, including left-handed Z-DNA, poly(dA) duplex (A-motif) and DNA bearing T–Hg–T base pairs. Their favorable thermodynamic stability under physiological conditions further ensures their application in cellular environments.

**Structural regulation of individual DNA nanostructures**

With the help of precise addressability and programmability, 2D or 3D nanostructures with different sizes and geometries have been deliberately designed. When stimuli-responsive noncanonical nucleic acids are incorporated into the molecular structure of these DNA nanostructures, various external stimuli can be used to regulate their structural properties. Specifically, they are subjected to transition among two or more distinct structural states in which the spatial arrangement of their structural components is nonidentical. This structural regulation leads to nano- or macroscale changes in their morphologies, which can be unambiguously visualized by imaging techniques. The mechanical force generated in this process also realizes internal movement in individual nanostructures, which is useful for constructing actuators implementable within the DNA nanotechnology toolkit.

Here, we highlight two research areas in the structural regulation of individual DNA nanostructures, including regulation of the distance and direction between specific components at the nanoscale and the spatial organization of non-DNA molecules tethered to the DNA nanostructure, which may lead to extraordinary functional nanodevices.

**Dynamic structural regulation of DNA nanostructures**

The fast-growing field of DNA nanodevices, which utilize DNA nanostructures to localize, guide, and regulate biomaterials at the nanometer scale, is an emerging research area. The structural reconfiguration of DNA nanostructures could directly and substantially influence their material properties. Noncanonical nucleic acids are powerful tools in transforming their conformations to generate mechanical force, thereby regulating the spatial relative distance and position of components of DNA nanostructures.

When noncanonical DNA of intramolecular conformation is embedded between two bound subunits in a DNA nanostructure, they can function as distance or angle adjusters by transitioning between the folded and unfolded states to execute motions. For example, the i-motif is compact and rigid in its folded state, with a width of ~0.8 nm, while in the open state, it is a single strand of DNA with an end-to-end distance that is a few nanometers longer. This property was utilized to measure motions between two origami units both in solution and on the surface. Specifically, after accommodating multiple (CCCTAA)₃CCC strands in parallel at the seam of...
two bound origami, ~3 nm variation in distance was observed by subjecting the mica surface first to a pH 7.2 buffer and then a pH 5.5 buffer, and vice versa (Fig. 3a).

Similarly, the curvature of the DNA nanostructure could also be reversibly altered by incorporating a monomeric noncanonical nucleic acid in the middle of duplex scaffolds. For example, a DNA origami nanoarm was constructed with 19 repeats of a tension-adjustable module, each comprising, a stem, piers, and bridge strands (Fig. 3b). The bridge strands connecting the piers acted as entropic springs to apply tension to bend the entire nanoarm. Because a G-quadruplex-forming sequence was incorporated into the bridge strand, it contracted in the presence of K+, thereby leading to the bending of the module and subsequently the deformation of the entire shape from the original linear shape into an arched shape in a cumulative fashion.

Relative movements between components of a DNA nanostructure lead to alterations in volume when it bears a cavity in its structure. This feature was readily illustrated by the DNA tetrahedron, as it is probably the simplest and most accessible 3D DNA cage. By inserting a single-stranded i-motif-forming sequence into one of the edges of a DNA tetrahedron immobilized on a gold surface, our group demonstrated proton-driven uptake and extrusion of water and ions in/from the tetrahedron inner cavity, resembling the expansion and compression of a water pump (Fig. 3c). The weight change of entrapped water caused by the conformational switching of the surface-confined DNA tetrahedron was measured using a quartz crystal microbalance (QCM). When PBS buffers with pH values of 8.0 and 5.0 were alternatively pumped into the QCM chamber, reversible conformational switching could be monitored by the change in both the frequency (blue) and energy dissipation (red) curves acquired with the quartz crystal microbalance.

Sensing is another important application field of reconfigurable DNA nanostructures modified with noncanonical DNA. DNA nanostructures of different shapes and compositions are able to serve as scaffolds or carriers for noncanonical DNA-based sensors in specific working scenes both in vivo and in vitro. The intrinsic dependence on protonation in the folding of the DNA triplex and i-motif endows them with the power to sense pH variations. Cells and organelles elaborately maintain appropriate proton gradients to function properly, and abnormal pH alterations have a substantial influence on multiple important cellular processes, such as apoptosis and cell proliferation, which lead to the development and progression of cancer.

Gaining knowledge of pH alteration and the exact value in biological media will therefore contribute to the understanding and
interference of these processes. Moreover, biosensors with G-quadruplexes as the target capture elements are able to detect potassium ions, which play a significant role in controlling cellular osmotic pressure and whose concentration abnormalities in blood are associated with heart disorders and lethal complications. In addition, G-quadruplex-forming oligonucleotides comprise a large group of aptamers that recognize ions (Cu$^{2+}$, Pb$^{2+}$, etc.), small molecules (ATP, ochratoxin A, etc.), and proteins (thrombin, nucleolin, immunoglobulin M, etc.).

A straightforward approach to functionalizing DNA nanostructures with the sensing ability is to integrate a monomeric triplex/tetraplex into their structures, with fluorophore-quencher or FRET pairs modified at its two terminals, so that the generation of a fluorescent signal and its relative intensity will exhibit dependence on the conformational transition of the integrated triplex/tetraplex. In a recent report, a DNA triangular prism was self-assembled to include i-motif-forming sequences in its three edges, and three identical FRET pairs were modified on both ends (Fig. 3d). The FRET signal was activated when the DNA prism entered acidic organelles, owing to intramolecular i-motif folding, which brought the FRET pairs into close proximity, while in neutral pH conditions, the DNA prism extended and diminished the FRET effect. High contrast and spatiotemporal pH mapping were thus achieved during endocytosis. In addition, instead of placing recognition elements directly between FRET pairs, another approach places the G-quadruplex-forming strand at a distance from the FRET pair on tunable DNA origami to regulate K$^+$-induced signal generation.

Artificial DNA nanostructures, including tetrahedral DNA, SNA, and DNA origami, have been employed recently as scaffolds to fabricate G-quadruplex-containing-aptamer-based sensors and drug delivery vehicles. For example, in the design of an electrochemical sensor for thrombin detection, the binding of target thrombin to its G-rich aptamer, which relies on a G-quadruplex structural transformation, opened hairpins conjugated on a polymeric ferrocene nanoparticle, releasing a single-stranded oligonucleotide that disassociated the DNA duplex, leading to the reformation of the DNA tetraplex. RNase A was encapsulated inside the cavity, and its enzyme activity was found to be reversibly controlled via pH-dependent opening and closing of the DNA tetrahedron because the outer DNA cage limited its exposure to substrates.

Another feature of controlling enzyme activity via spatial regulation was demonstrated by using a DNA tetrahedron. Specifically, in the design of an opening/closing-adjustable DNA tetrahedron, an i-motif and its partially mismatched complementary sequence constituted one of the six duplex edges, which disassembled at pH 6.4, thus opening the tetrahedron, and rehybridized at pH 7.3, leading to the reformation of the tetrahedron. RNase A was encapsulated inside the cavity, and its enzyme activity was found to be reversibly controlled via pH-dependent opening and closing of the DNA tetrahedron because the outer DNA cage limited its exposure to substrates.

In addition to controlling enzyme activity, the reconfiguration of three-dimensional DNA objects can be utilized to develop smart, targeted drug delivery systems that can respond to stimuli present in living organisms. For
instance, a DNA origami nanocapsule, with two capsule halves at one edge bearing sticky ends that are capable of associating into a bimolecular triplex, was demonstrated to undergo reversible opening/closing controlled by a sharp pH change, achieving the encapsulation and display of macromolecular payloads (Fig. 4c)\(^64,65\).

**Dynamic regulation of hierarchical DNA nanostructures**

Recently, research on the programmed assembly of hierarchical DNA architectures has attracted increasing attention\(^66\). The emergence of different strategies to combine preformed structures into higher-order structures, such as sticky end association and blunt end stacking, broke through the limitation caused by the one-pot self-assembly method in scaling up both the size and complexity of DNA nanostructures\(^67\). However, these higher-order structures are inert, and the relative positions of their components are fixed once they are fabricated, which prevents their use in practical applications where reversible assembly/disassembly is critical\(^13\). In this case, noncanonical nucleic acids provide a means to induce not only the assembly/disassembly of DNA nanostructures but also rearrangement of the relative positions of the structural units\(^33\). This could be achieved in the sequence design stage by integrating the two strands of an intermolecular triplex/tetraplex conformation in the sticky ends protruding from the DNA nanostructures or by utilizing noncanonical DNA of intramolecular conformation and its partly complementary strand as the sticky ends. This form of dynamic regulation of hierarchical DNA nanostructures facilitates the development of intelligent DNA architectures, drug delivery systems, and molecular machines mimicking living systems to conduct conformational transitions in biomacromolecules to achieve specific functions according to the altered environment.

**Reversible assembly of hierarchical DNA nanostructures**

When multiple DNA nanostructures with tether strands comprising each of the two fragments of a bimolecular triplex/tetraplex are functionalized, they are able to transition reversibly between the aggregated and disaggregated states along with the hybridization and dissociation of the sticky ends\(^32,33\). Alternatively, by integration of a monomeric triplex/tetraplex-forming oligonucleotide in the sticky ends of one kind of DNA nanostructure and its partly complementary oligonucleotide in the sticky ends of another DNA nanostructure, the hybridization of the two types of sticky ends will bring together the two nanostructures, while the refolding of the triplex/tetraplex will lead to their separation\(^32,33\).

DNA origami was the most favorite substrate to employ to demonstrate the ability of a dimeric triplex/i-motif/G-quadruplex to assemble or disassemble multicomponent nanostructures by connecting or disconnecting individual structural units. For example, dimeric and monomeric origami structure transitions were conducted using an origami frame with a tether of ssDNA and another modified with a duplex (Fig. 5a)\(^32\). An 8-bp duplex (green) was incorporated in the design to stabilize the triplex structure.

In regard to applying a bimolecular DNA tetraplex to direct the assembly of DNA nanostructures, two means of adding directionality to their hybridization have been proven to be successful: appending a short duplex to the tetraplex or replacing the two loops of the tetraplex with well-designed hairpins. For instance, as shown in Fig. 5b,
the alternating addition of $K^+$ and cation chelating agent 18-crown-6-ether will control dissociation and reassembly of the origami dimer. The formation of the bimolecular G-quadruplex relied on the assistance of the short duplex (purple and cyan in the figure); otherwise, any two of the G-rich strands would hybridize and afford a mixture of disordered origami dimers.

Another example employed bimolecular G-quadruplexes modified with hairpins as two sets of sticky ends to perform the dimerization and cyclization of a triangular DNA nanostructure (Fig. 5c). In this instance, two G-rich strands with different stabilities, G5GCGGAAGGCG (abbreviated as G5-4GC, red), and G5GCGGAAGGCG (abbreviated as G5-4GC, red), were integrated on two vertexes of the DNA nanotriangle. With higher thermal stability and faster folding kinetics, G5-4GC folded in advance of G4-4GC, leading to the first formation of a bowknot-like structure and then cyclization. Unmodified G-rich sequences were not robust enough to induce the dimerization of the DNA triangles under the given conditions. Subjecting the cyclic product to a $K^+$ chelator 18-crown-6 led to the reformation of the nanotriangle monomer.

In addition to dimerization, stepwise multimerization of DNA nanostructures could be achieved by incorporating multiple sets of sticky ends containing noncanonical nucleic acids. The sequential utilization of two triplex-duplex conformational switching elements differing in base compositions was implemented to reversibly control the multimerization of DNA origami, mimicking living systems to assemble functional macromolecules in response to certain stimuli in a stepwise and reversible fashion. The pH sensitivity of the triplex depended strongly on the relative content of CGC/TAT triplets in the sequence. For instance, by increasing the TAT content from 0 to 100%, the observed pKa for a 10-bp-stem triplex was increased from 6.5 to 10.2. Thus, the regulation of triplex pH sensitivity over a wide window of pKa variation (~5 pH units) by manipulating the relative content of CGC/TAT triplets provided a means to realize the
hierarchical fabrication of complicated and/or large DNA structures reversibly and selectively in response to multiple steps of environmental stimulation, which would not be possible by duplex formation alone. In an example, origami with triplexes of varying pKa as linkers was subjected to transition among the monomer, dimer, and trimer states in a stepwise fashion in response to three different pH environments. This strategy was further adopted in the stepwise construction of nine-unit DNA origami clusters (Fig. 5d).

In addition to DNA origami, other forms of nanostructures (DNA tetrahedron, SNA, etc.) can also be readily constructed into reversible hierarchical assemblies with noncanonical nucleic acids. Since the DNA tetrahedron was predominantly confined in the acidic organelle lysosome after endocytosis, it is reasonable to implement the DNA tetrahedron in applications related to the acidic medium in lysosomes (pH 4.5–5.0), such as imaging biomolecules present exclusively in lysosomes. A recent study achieved lysosome-specific ATP imaging by implementing an AND-logic gate, with H⁺ and ATP as inputs, on DNA tetrahedrons. As depicted in Fig. 6a, one-half of a dimeric i-motif and one-half of a dimeric ATP aptamer were tethered to the vertexes of two tetrahedrons so that once the tetrahedrons entered acidic lysosomes, they assembled into a heterodimeric architecture by forming an intermolecular i-motif, thereby enabling proximity-induced ATP aptamer folding and FRET activation in response to endogenous ATP.

Moreover, the weak acidity (pH 6.4–6.9) of the tumor microenvironment becomes a valuable target in the design of controlled drug delivery systems. SNA assemblies loaded with antitumor drugs are able to release them by disaggregation in response to the acidic milieu. For example, a deep-penetrating drug delivery system was developed by employing stepwise triplex-duplex and i-motif-duplex conformational switching, to direct the disaggregation of SNAs (Fig. 6b). Specifically, gold nanoparticles with a diameter of ~15 nm were loaded into the pores of mesoporous silica nanoparticles (diameter ≈ 150 nm) via triplex-forming sticky ends, and C-rich sticky ends in their duplex conformation embedded the anticancer drug doxorubicin into the surface of SNAs. When this type of drug carrier was administered, it first accumulated in the tumor region via enhanced permeability and retention effects. Subsequently, the slightly acidic tumor microenvironment (pH ≈ 6.5) induced the structural transition of triplex DNA, leading to the release of SNAs from the silica nanoparticle pores to the deep region of the tumor tissue, because of their small size. After the SNAs were internalized into the tumor cells, the acidic endosomal pH (ca. pH 5.0) promoted the folding of the i-motif, which led to the final release of doxorubicin. This dual pH responsiveness based on the cooperative conformational switching of two types of noncanonical nucleic acids prompted the successful two-step disassembly of novel SNA-containing nanoparticles in vivo.

Spatial rearrangement of hierarchical DNA nanostructures

Taking advantage of the stimuli responsiveness of noncanonical nucleic acids, not only the controlled and reversible assembly of DNA nanostructures, but also their spatial rearrangement could be achieved. The two conformational transition strategies, namely, intermolecular-
More complicated reconfiguration of origami assemblies was realized by using four sets of sticky ends, two of which were able to switch between an intermolecular tetraplex and a random coil (e.g., dimer DA and dimer BC in Fig. 7b), while the other two, between an intramolecular tetraplex and a duplex (e.g., dimer AB and dimer CD)\textsuperscript{33}. The alternating addition of K\textsuperscript{+} and cation chelating agent 18-crown-6-ether controlled the dissociation and reassembly of the origami dimer. This type of stimulation-mediated morphological interconversion laid the foundation of the ‘origami chemistry’ concept introduced by Willner’s group, which considers individual origami tiles as reaction elements to be subjected to synthesis, decomposition, single replacement, and double replacement. Well-designed noncanonical nucleic acid sequences were integrated in the protruding tethers on the tiles to connect and disconnect reaction elements on demand. The reconfiguration of the AB + CD dimer mixture into BC + DA shown in Fig. 7b resembles a double-replacement reaction. Moreover, a switchable catalytic function was engineered by introducing sequences of Mg\textsuperscript{2+}-ion-dependent DNAzyme and hemin/G-quadruplex DNAzyme in the bridge.

**Conclusions**

The past few years have seen the intriguing interweaving of noncanonical nucleic acids with artificial DNA architectures. In this review, we highlighted different strategies for subjecting noncanonical nucleic acids to conformational transitions and their utilization in regulating DNA nanostructures for nanomedical applications. The specific roles of both entities were examined in every surveyed example. The incorporation of noncanonical nucleic acids into predesigned DNA nanostructures renders successful manipulation of the morphological alteration, movement, and assembly/disassembly processes of static nanostructures, exhibiting potential in fabricating biomimetic materials, molecular motors, and controlled drug delivery systems.

With the accumulation of experience in integrating noncanonical nucleic acids into DNA architectures, some previously neglected issues have come to light. For example, the stability of noncanonical nucleic acids varies to a noticeable extent after they are incorporated into DNA nanostructures\textsuperscript{71,72}. Careful examination of their thermodynamics and kinetics is thus recommended when they become components of a larger structure. Additionally, the exact folding topology of DNA tetraplexes has rarely been taken into consideration in most designs, probably on account of their complexity. Nonetheless, differences in topology may influence the morphology, thermodynamics, and kinetics of the synthesized DNA nanostructure\textsuperscript{27}. A deeper understanding of the correlation between the DNA
primary structure and its folding topology will prompt the utilization of DNA tetraplexes with a single topology in delicate devices.\(^{27,42}\)

Although various studies have been carried out to examine the ability of noncanonical nucleic acids to transform artificial DNA nanostructures, there remains a need for a new approach to regulate the motion and assembly process of the existing systems. It is highly expected that more manipulation capabilities will come from introducing small molecule ligands or proteins that specifically interact with noncanonical nucleic acids, or from introducing appropriately chemically modified nucleosides\(^{73}\), as well as mammade DNA analogs (locked nucleic acids, unlocked nucleic acids, peptide nucleic acids, etc.)\(^{74,75}\).

The regulatory capacity of noncanonical DNA on DNA nanostructures holds promising prospects in the fields of early cancer diagnosis and synthetic biology.\(^{76}\) For example, a two-state cage-like DNA nanostructure, which can be opened and closed by noncanonical DNA, could serve as a platform for the capture and release of diagnostic biomarkers (e.g., microRNA, circulating tumor DNA, proteins, and extracellular vesicles) and for circulating tumor cells\(^{77}\) in a repeatable and on-demand manner. When the DNA nanostructure is immobilized on the interface of either a solid or soft material (e.g., gold surface, microfluidic chip, or hydrogel), these biomarkers could be extracted from body fluids and then enriched for further analysis. On the other hand, living cells can be conjugated with artificial DNA architectures\(^{78}\), and it is therefore possible to expand the spatial arrangement or assembly/disassembly information of the DNA architectures to cells. This would be advantageous to the engineering of cellular morphology and intercellular communication.\(^{79}\)

Overall, the richness of nucleic acid conformational polymorphisms, the versatility of artificial DNA nanostructures, and the interweaving of the two groups synchronously contribute to the growth of DNA-related science and technology, generating an ever-increasing ability to perform manipulation at the nanoscale. The resultant opportunities in developing biomimicking materials, molecular motors, drug delivery systems, and biosensors hold great promise for translating DNA materials and devices from the laboratory to the clinic in the near future.

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Conflict of interest

The authors declare no competing interests.

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