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Mechanically Interlocked DNA nanostructures for Functional Devices

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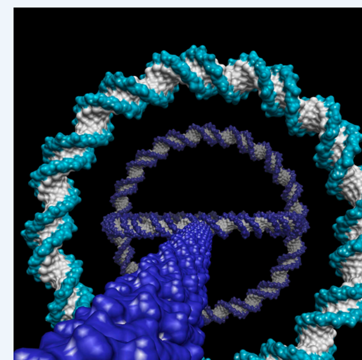
CONSPECTUS: Self-assembled functional DNA oligonucleotide based architectures represent highly promising candidates for the creation of nanoscale devices. The field of DNA nanotechnology has emerged to a high level of maturity and currently constitutes one of the most dynamic, creative, and exciting modern research areas. The transformation from structural DNA nanotechnology to functional DNA architectures is already taking place with tremendous pace. Particularly the advent of DNA origami technology has propelled DNA nanotechnology forward. DNA origami provided a versatile method for precisely aligning structural and functional DNA modules in two and three dimensions, thereby serving as a means for constructing scaffolds and chassis required for the precise orchestration of multiple functional DNA architectures. Key modules of these will contain interlocked nanomechanical components made of DNA. The mechanical interlocking allows for performing highly specific and controlled motion, by reducing the dimensionality of diffusion-controlled processes without restrictions in motional flexibility.



Examples for nanoscale interlocked DNA architectures illustrate how elementary functional units of future nanomachines can be designed and realized, and show what role interlocked DNA architectures may play in this endeavor. Functional supramolecular systems, in general, and nanomachinery, in particular, self-organize into architectures that reflect different levels of complexity with respect to their function, their arrangement in the second and third dimension, their suitability for different purposes, and their functional interplay. Toward this goal, DNA nanotechnology and especially the DNA origami technology provide opportunities for nanomechanics, nanorobotics, and nanomachines. In this Account, we address approaches that apply to the construction of interlocked DNA nanostructures, drawing largely form our own contributions to interlocked architectures based on double-stranded (ds) circular geometries, and describe progress, opportunities, and challenges in rotaxanes and pseudorotaxanes made of dsDNA.

Operating nanomechanical devices in a reliable and repetitive fashion requires methods for switching movable parts in DNA nanostructures from one state to another. An important issue is the orthogonality of switches that allow for operating different parts in parallel under spatiotemporal control. A variety of switching methods have been applied to switch individual components in interlocked DNA nanostructures like rotaxanes and catenanes. They are based on toehold, light, pseudocomplementary peptide nucleic acids (pcPNAs), and others. The key issues discussed here illustrate our perspective on the future prospects of interlocked DNA-based devices and the challenges that lay ahead.

INTRODUCTION

Nanomachines of a future nanotechnology era are proposed to consist of complex device architectures that are hierarchically assembled from a diversity of functional compartments, each of which consists of elementary subunits that are capable of performing highly specific tasks.[1] A generalized guess is that these modules, in analogy to machines in the macroscopic world, will contain moving parts as essential components, driven by power conversion units, but also switches, electronic building blocks, and so forth. Eventually, all these units will be mounted on a rigid backbone, similarly to the chassis of a car, so that different units can work in lockstep to perform the intended action. This hierarchical design strategy is illustrated in Figure 1. A highly promising design strategy relies on mimicking the composition of macroscopic machines and on finding analogs in the related nanoscale world. Detailed knowledge is required about (i) how individual functional units may be designed from nanoscale building blocks to perform designated elementary operations, (ii) how their individual actions can coalesce to a complex functionality, (iii) how this functionality can be adapted to match requirements in various fields of application, and (iv) how the yields of DNA nanostructures can be increased to enable DNA nanotechnology for practical applications (an important step toward this goal was recently achieved[2]).

These ambitious efforts need to include many aspects of all scientific disciplines that are involved in studying nanoscale systems, such as the natural, engineering, and medical sciences. Concepts from mechanical engineering will be transferred in the ongoing creation of nanotechnology devices, and well-known design strategies can be utilized as role models. Meanwhile, fundamental building blocks for potential nanomechanical device units that provide a controllable functionality will eventually be involved in the aforementioned complex device architectures. Design strategies relying on building blocks at the

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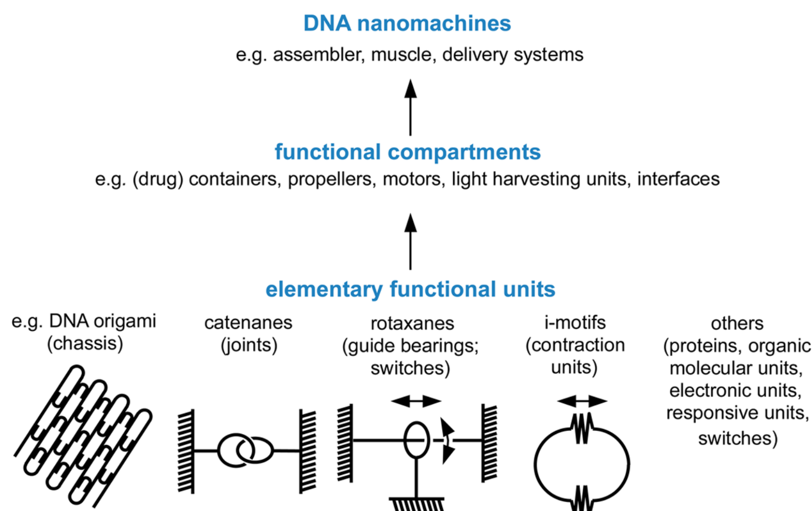

Figure 1. Hierarchical assembly of complex DNA nanomachines from functional compartments. These will consist of elementary units, each featuring a highly specific functionality.

atomic, molecular, or supramolecular size level are currently under evaluation for developing functional devices and learning about the required interplay to obtain the essential level of complexity.

The advent of nanoscience yielded an increased understanding about matter beyond the pure description of ensembles. Organic chemistry has provided a plethora of chemical reactions relying on the construction of individual molecules that also form supramolecules. Each molecular (sub)unit includes a high structural uniqueness and information density with respect to its designated structure, bond type, and (hetero)element composition.[3,4] Moreover, naturally evolved biological systems provide highly complex, yet well-defined structural and functional units that could also serve as autonomously functioning building blocks in biohybrid nanostructures.[5] The challenge now is to efficiently combine synthetic units with these biological components without loss of functionality.

"Hard matter" examples from inorganic and organic chemistry rely on covalent bonds, usually formed by irreversible processes. Conversely, most examples for reversible bond formation rely on noncovalent interactions. Here the sum of plenty of "weak" bonds (hydrogen-bonds, π-stacking interactions, van der Waals forces) is essential for supramolecular self-assembly. The latter relies on equilibrium processes between related building blocks that are initially arranged in a random fashion to spontaneously arrange into highly ordered "superstructures". Reorganization of molecules and submolecular units includes many degrees of freedom and extends over a broad energy scale.

DNA is increasingly used as a construction material for synthetic functional devices combinable with most synthetic and biological building blocks and can form various structural elements. Even the B-type DNA double helix can be employed for constructing highly complex DNA nanoarchitectures and functional devices. Initiated by Nadrian Seeman in the 1980s,[6] then continuously developed further,[7,8] and again boosted by Paul Rothemund's invention of DNA origami,[9] the past decade has witnessed an exploding complexity of structural and functional DNA nanostructures. A driving force for these DNA nanoarchitectures is a programmable self-organization procedure that relies on double strand formation on one hand, and structural units (bendings, kinks) on the other hand. The latter result from clever employment of fragments of helix structures, mismatching base pairs, and crossover sequences in which the sequence of one single strand region matches sequence intercepts from two or more additional single strands. This has led to a key requirement of a detailed understanding about how the DNA architectures depend on the DNA base pair sequence, how architectural DNA motifs can be assembled from scratch, and how functionality can be implemented (Figure 1). Because the DNA fragments used for this purpose are mostly of synthetic origin, the implementation of classical-organic-chemistry modifications is straightforward. Similarly, their combination with proteins, ribo-/DNAzymes, or aptamers to yield biohybrid nanostructures can be achieved by various strategies. In any case, shape-persistence of DNA-based structures has to be achieved by delicate design strategies to overcome the otherwise high degrees of freedom that prohibit mechanical energy transfer within the device.

A breakthrough in DNA nanotechnology came with the advent of DNA origami.[9] These DNA origami form spontaneously upon mixing short single stranded (ss) DNA staples that hybridize to different regions of one long ssDNA and can be viewed as programmed supramolecular architectures where the program code is stored in the base sequence of each ssDNA unit.

DNA origami have been used for various purposes in DNA nanostructures, for example, as a "ruler" in super-resolution optical microscopy,[10] as chassis for the attachment of ssDNA to gold nanoparticles,[11] to create plasmonic hot spots for surface-enhanced Raman spectroscopy (SERS).[12] Mask lithography allows the prestructuring of large surfaces to form a grid of binding sites for DNA origami that can assemble on the patterned surfaces.[13] When functionalized to bind additional particles or units, the highly precise arrangement of DNA nanomachinery should become straightforward. Moreover, DNA origamis have undergone a transition from two-dimensional (2D) to three-dimensional (3D) structures. For example, 2D DNA origami sheets have been functionalized to carry ss regions at their sides to yield a "DNA box" forming the six sides of a cube where one side could be switched open and close like a lid.[14]

INTERLOCKED DNA NANOSTRUCTURES BASED ON CIRCULAR GEOMETRIES

Coming originally from aptamer research and supramolecular chemistry, we entered the field of DNA nanostructures by

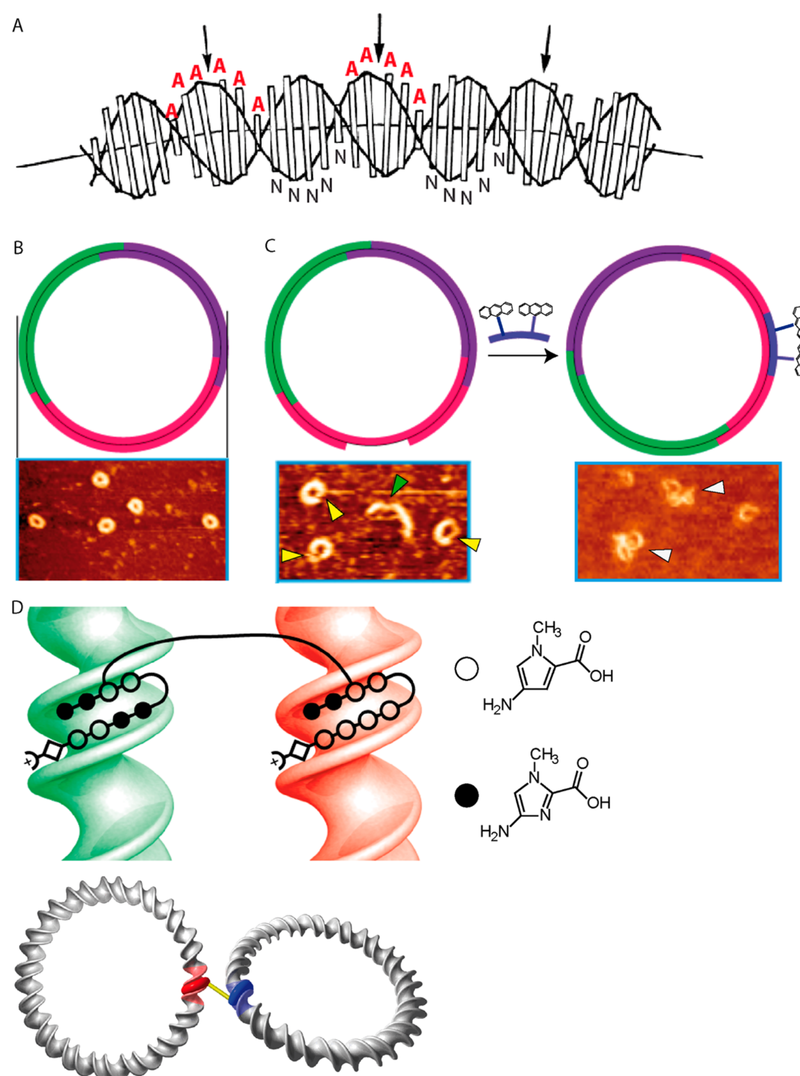

Figure 2. Generation of dsDNA nanorings. (A) Intrinsic dsDNA-bending by repetitive A-tracts. (B) 168-bp dsDNA-nanoring ligated from three 51-mer dsDNAs, flanked by 5-nt ssDNA overhangs (top) exhibit a regular, nondistorted shape (bottom). Adapted with permission from ref 20. Copyright 2008 Wiley-VCH.[20] (C) Assembly of dsDNA nanocircles into oligomeric aggregates mediated by anthracene intercalation.[25] Adapted with permission from ref 20, Copyright 2008 Wiley-VCH; and ref 25, Copyright 2010 RSC Publishing. (D) Polyamide struts for defined assembly of two dsDNA nanocircles.[21]

utilizing intrinsically bent double-stranded (ds) DNA fragments to synthesize dsDNA nanocircles for assembling interlocked DNA nanostructures. Mechanically interlocked DNA architectures consist of two or more components that are connected to one another by interlocking rather than by chemical bonds. Thereby, while the components can move relatively to one another, they are unable to dissociate. These properties suit interlocked DNA nanostructures as highly versatile elementary functional units in dynamic DNA nanotechnology, where the future goal is to build artificial nanomachines that perform tasks of similar complexity as biological machines. The assembly of interlocked DNA and RNA nanostructures such as catenanes,[15,16] borromean rings,[17] trefoil- and other knots[15,18] from ss nucleic acids had already been achieved. Many of these systems represent experimental and intellectual masterpieces. However, we sought to employ dsDNA nanocircles that are shape persistent as compared to previously used ssDNA that fold into hardly predictable, often complex, 3D structures. dsDNA nanocircles containing repetitive, intrinsically bent AT-tracts (Figure 2A)[19] hardly exhibit any ring strain, and are advantageous in various respects when assembling functional interlocked DNA nanostructures. By ligating synthetic 21-base ds-precursors, circular oligomers with 105, 126, 147, 168 (from five, six, seven, eight 21-mers), or more base pairs (bp) are obtained. However, these dsDNA nanocircles were not amenable to functionalization due to the unchangeable DNA sequence. This caveat was overcome by synthesizing three AT-tract-containing 51-mer dsDNAs, flanked by 5-nt ssDNA overhangs for ligation. Ligation of three of them led to 168-bp ds nanorings, which allowed leaving a ss 21-nucleotide long region in one of the 51-mers without loss in efficiency of ligation (Figure 2B). The sequence of this ss-gap region could be chosen ad libitum, aiming for a guided hybridization of complementary oligodeoxynucleotides (ODNs).[20] Atomic force microscopy (AFM) analysis confirmed the uniformly circular structure of the entirely ds macrocycles and also showed that the 21-mer ss-gap that lacked poly-A tracts did not show any ring deformation (Figure 2C). These gap-containing nanorings provided versatile intermediates that could hybridize to any desired functionalized ODN to access small circular dsDNAs with desired properties.

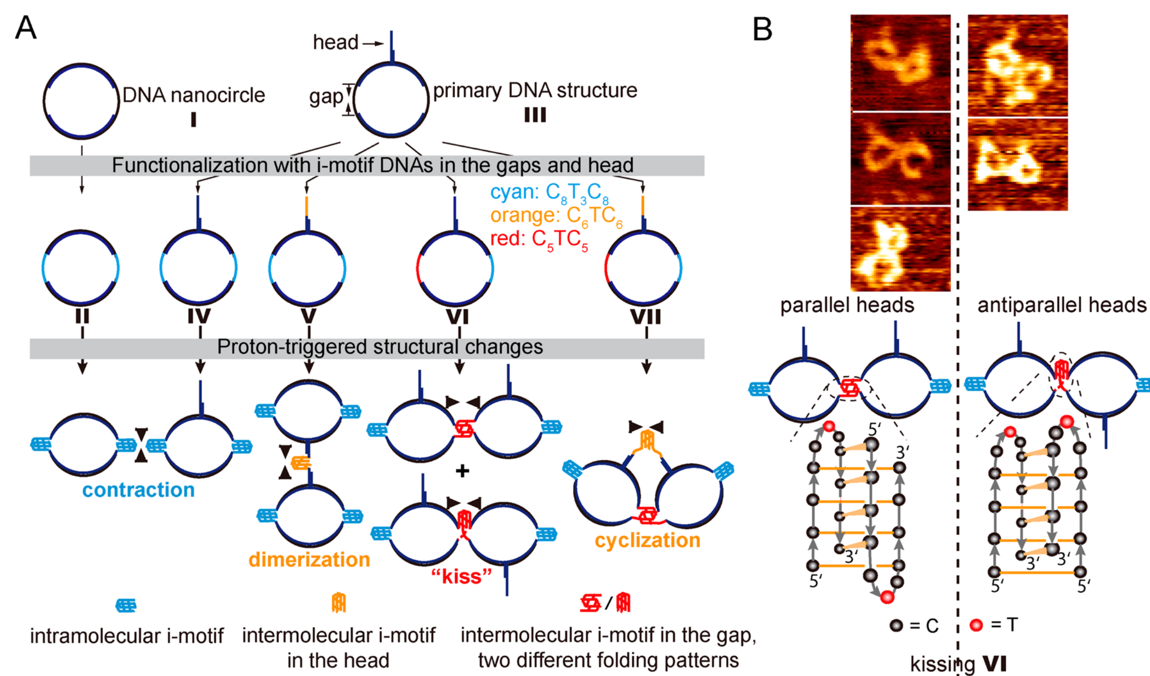

Figure 3. i-Motif-programmed functionalization of DNA-nanocircles.[26] (A) DNA-nanocircles with intra- and/or intermolecular i-motif DNAs in the ss-gap regions and their expected H^+-triggered structural changes. Cyan, unimolecular i-motif; red, orange: two bimolecular i-motifs. (B) AFM images of the DNA structure VI at pH 5.0, demonstrating that two monomers are "kissing" in either a parallel or an antiparallel manner. Adapted with permission from ref 26. Copyright 2013 American Chemical Society.

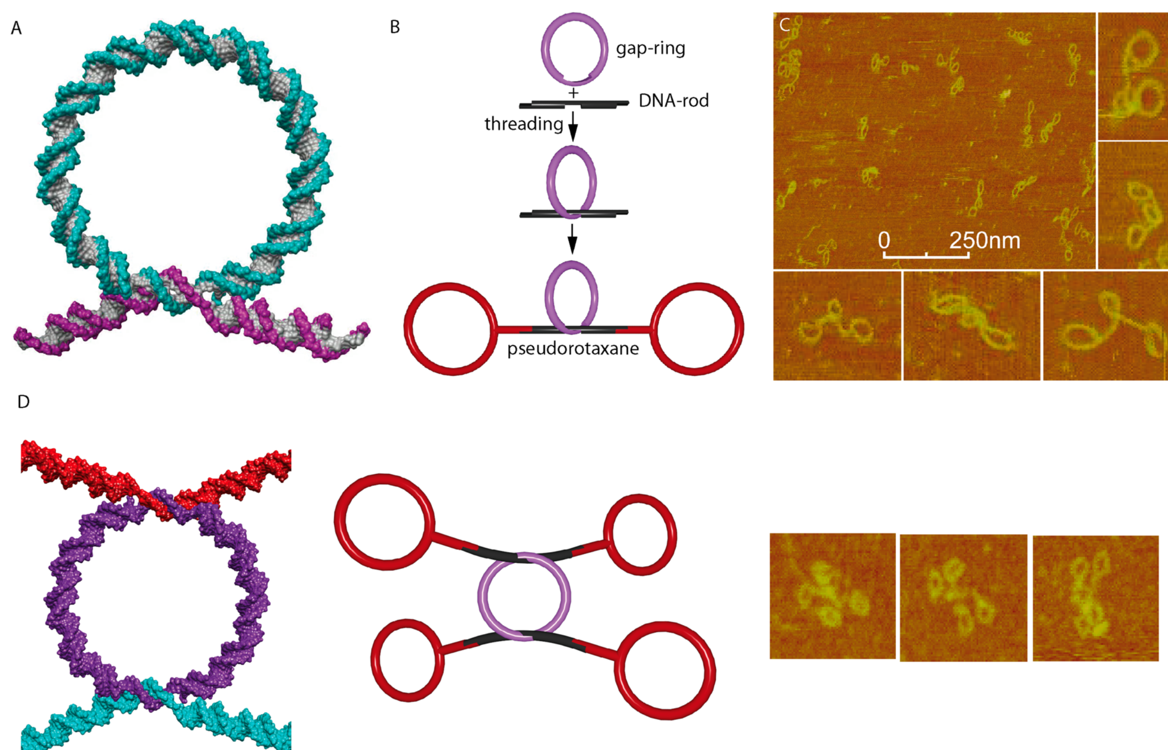

Figure 4. (Pseudo)rotaxane assembly.[28] (A) Threading principle of a ss-gap-containing dsDNA-nanocircle with an axle containing a complementary gap. (B) Schematic for the assembly of a pseudorotaxane from a 126-bp macrocycle and 168-bp rings at the termini. (C) AFM images of these pseudorotaxanes. (D) Schematic for the threading principle and AFM images of a [3]pseudorotaxane. Adapted with permission from ref 28. Copyright 2010 Nature Publishing Group.

Initial proof-of-principle applications of such DNA-nanorings included the introduction of molecular recognition modules, for example, for a defined complexation of two or more of these circular building blocks in a clearly defined and sequence-specific fashion. First, we employed synthetic DNA-binding polyamides containing *N*-methyl imidazole and *N*-methyl pyrrole residues to bind sequence-specifically by reading out sequence information in the minor groove of a B-type dsDNA (Figure 2D).[21]

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Second, the formation of a dumbbell-shaped complex between two nanorings was achieved by hybridizing each gap to complementary RNA sequences to form a three-way junction of a mixed RNA/DNA assembly.[22] Two RNA sequences terminated in hairpin loop secondary structures, which interact in a specific "kissing-loop" complex via noncanonical interactions in the presence of Mg^{2+}-ions.[23,24] Finally, the employment of simple two-armed branched ODNs ligated to the ss-gap of DNA-nanocircles also led to dumbbell-shaped architectures.[25] These represented the first example of an entirely ds subtype of a DNA nanostructure in which linear dsDNA connects two dsDNA nanocircles through three-way junctions.

We recently expanded the guided assembly of DNA-nanorings to scaffolds of different shapes[26] to employ various intra- and intermolecular i-motif DNAs for the functionalization of ss-gaps, and a branched-out "head" structure of dsDNA nanocircles. We systematically studied the folding of i-motif DNAs with two C-tracts as a function of C-tract length. Two stretches of six or less C residues, such as C_6TC_6 and C_5TC_5, favor the intermolecular i-motif formation, while longer C-tracts like $C_8T_3C_8$ formed intramolecular i-motif structures with unusually high thermal stability. When incorporated into the ss-gap, the intra- and intermolecular folding of i-motif DNAs could be controlled in response to pH change and by adjusting the length of C-tracts. The structural changes observed under acidic conditions included DNA-nanocircle contraction, dimerization, kiss, and cyclization (Figure 3A), as verified by gel electrophoresis and AFM. Thereby, two different folding patterns of a bimolecular i-motif could be visualized (Figure 3B). The study demonstrated how to harness the controllable intra- versus intermolecular folding of i-motif DNAs for the functional programming of DNA-nanocircles. Previously, we had also employed pH- and K^+-triggered structural interconversions of i-motifs, G-quadruplexes, DNA-triple-helices, and DNA duplexes for performing molecular logic operations such as NOR, INH, or AND gating.[27] Combining insight from both studies will now yield pH-driven, reversibly switchable dsDNA nanocircle for programming the dynamic topologies of interlocked systems, and to operate individual units in DNA nanomechanics.

ROTAXANE AND PSEUDOROTAXANE NANOSTRUCTURES MADE OF CIRCULAR DNA

Gap-containing DNA-nanorings are perfectly suited for hybridizing with a complementary ss sequence as part of a linear DNA, flanked by dsDNA. If, by design, hybridization can only occur when the linear DNA threads through the nanoring (Figure 4A),[28] a pseudorotaxane, a rotaxane precursor, will form. Our first example employed a 126-bp macrocycle with part of the ss region in the axle and rigid 168-bp dsDNA nanorings as termini (Figure 4B). High-resolution AFM of these pseudorotaxanes even allowed distinguishing between the smaller macrocycle and the larger terminal rings (Figure 4C).

A genuine rotaxane was obtained from these structures by adding short ss ODNs in a toehold-like approach.[28] This forces the macrocycle and the axle to dehybridize, resulting nominally in a genuine rotaxane. Here, it turned out that the macrocycle dethreaded from the dumbbell by slowly slipping over the stopper rings. The rotaxane lifetimes strongly depended on the radial ratios of the stopper-rings and the macrocycle that could be mechanically fine-tuned between 15 min and >3h. A sufficiently high lifetime was obtained with a [3]rotaxane consisting of a 126-bp macrocycle and two 126-bp dumbbells threaded through it (Figure 4D; lifetime > 48 h). These dsDNA rotaxane systems showed a clear correlation between stopper size, macrocycle-crowding, and dethreading time and in this way behaved similar to synthetic rotaxanes from macromolecular chemistry. Potential applications include time-dependent logic gating, signal generation, and molecular switching.

To create a truly long-term mechanically stable rotaxane, we moved into the third dimension by synthesizing spherical stoppers in which two 168-bp nanorings cross each other by forming Holliday junctions at the poles of the resulting spheres (Figure 5A). These stoppers prevented the macrocycle from dethreading in case of the 126-bp macrocycle—even after a week at ambient temperature, no disassembly could be detected. However, a 168-bp macrocycle dethreaded fully within 30 min. Free mobility along of the macrocycle along the axle was proven by labeling the macrocycle with a fluorescence probe and a proximal site in the axle with a quencher dye: Only in the presence of the correct release-ODN dequenching was observed, and control experiments with scrambled versions of the release-ODN proved negative.

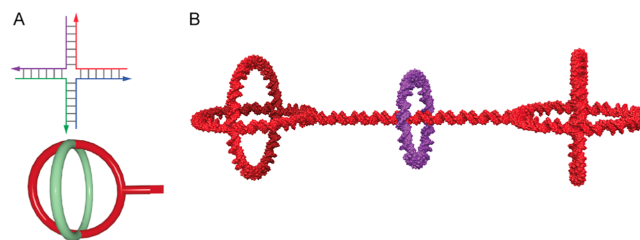

Figure 5. Mechanically stable dsDNA-rotaxane. (A) Spherical stopper containing 168-bp crossover rings with Holliday junctions at the poles. (B) Mechanically stable rotaxane with a 126-bp macrocycle.

This stable dsDNA rotaxane represented the first all-DNA rotaxane of its kind (Figure 5B). There are a few examples of biohybrid rotaxane architectures in which usually the macrocycles are made of protein while the axle is composed of DNA. For example, an α-hemolysin transmembrane pore was threaded by DNA-PEG hybrid strands to yield a functional rotaxane where the macrocycle was made of protein.[29] In a natural biohybrid rotaxane, the toroidal bacterial β clamp circumscribes a dsDNA, slides along the DNA, and anchors DNA polymerases to the template to allow highly processive replication without detachment of the polymerase.[30]

More recently, Itamar Willner and co-workers demonstrated the synthesis of DNA rotaxanes that are stoppered by 10 nm gold nanoparticles (Au-NP) attached to the 5′- and 3′-ends of an axle.[31] A largely single-stranded macrocycle was threaded over the axle and could hybridize to at least two implemented ss sites. By attaching Au-NPs that differed in size from the 10 nm stopper NPs and performing STEM and fluorescence quenching experiments, they showed that nucleic acids can be employed as fuels or antifuels, and different hybridization sites on the axle could be actuated by the macrocycle.

MECHANICALLY REINFORCED AXLES IN dsDNA ROTAXANES

One of our goals is to employ linear interlocked DNA nanostructures for converting the mechanical motion of the macrocycles along the axle vector into mechanical energy to transmit force in directed motion. Pyrophosphate hydrolysis, or molecule binding, fuels mechanical work in biological systems. In the case of our rotaxanes, this energy could be gained by

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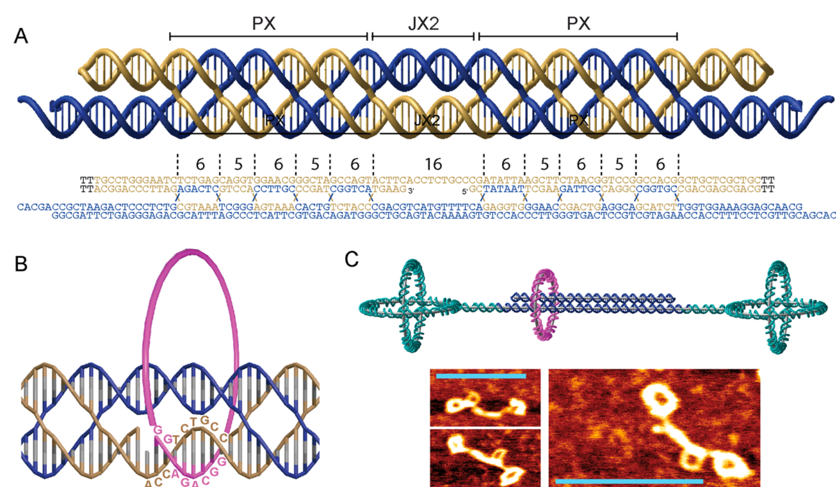

Figure 6. DNA rotaxane with mechanically reinforced PX100 axle. (A) Structure and sequence of PX and JX2 paranemic crossover DNAs used in the axle-design. (B) Threading-principle: The ss-gap in the macrocycle ensures that the middle of the hybridization site becomes located at an outer position. (C) AFM images of the dsDNA-rotaxane demonstrate the mechanical stiffness of the PX100 axle. The 126-bp macrocycle preferentially locates at the singly dsDNA-region in the axle.[34] Adapted with permission from ref 34. Copyright 2012 Wiley-VCH.

hybridization of ODNs. To achieve this, the DNA nanostructures require gradual advancements and possibly also their integration into more complex biohybrid nanomachinery. Evidently (Figure 4B), the mechanical properties of these pilot rotaxanes still have mechanical disadvantages with respect to persistence length: The axle exhibits a fairly high degree of flexibility and a momentum of the moving macrocycle along the axle simply leads to its undesired deformation due to the translational amplitude of ca. 100 bp in the axle. Avoiding this caveat is a task that is all but trivial! The axle requires a mechanical reinforcement, or an increase of persistence length, for example, by interweaving two or more DNA double-strands by multiple reciprocal strand exchanges, for example, in a "paranemic crossover" (PX) structure (Figure 6A). Ned Seeman and co-workers first established this type of DNA nanostructure in which reciprocal base pairing holds independent dsDNAs together.[32,33] However, the mechanical perfection of the PX-element somewhat contradicts with the threading principle required for interlocking the rotaxane architecture,[28] and achieving quantitative threading thus requires an entirely different design in various respects: First, any crossover at the 8-bp axle-gap/macrocycle-gap binding site had to be avoided. Second, this site had to be located at an outward position.

The axle and macrocycle design shown in Figure 6A,B considers these aspects.[34] For the axle, we designed two parallel dsDNA strands that are connected by six double crossovers, and implemented a so-called JX2 element in the center with an 8 bp ss-gap pointing outward of the entire PX100 axle. Consequently, hybridization to a macrocycle with a complementary ss-gap is possible only if the axle threads through the macrocycle. The PX100 axle contained sticky ends for ligating the stopper DNAs, and pseudorotaxane assembly was readily possible. Addition of the release-ODNs then led to a stable rotaxane with a reinforced axle that did not show any distortion in AFM analyses, demonstrating that axles based on paranemic crossover DNAs can serve as components for mechanical stabilization (Figure 6C). The study provided generally applicable design strategies for the incorporation of mechanically interlocked DNA architectures or even DNA origami structures. DNA rotaxanes with mechanically reinforced axles might serve as precursors for complex molecular machines capable of force transmission.

■ INTERLOCKED dsDNA NANODEVICES SWITCHED BY LIGHT

An attractive goal in functional DNA nanotechnology is reversibly controlling the shuttling of interlocked macrocycles between mobile and stalled states by photoregulation, for example, by incorporating azobenzene (AB) derivatives into the release-ODNs or the gap regions. At 440 nm, the planar *trans*-AB forms, which stabilizes the duplex structure and the release-ODN hybridizes to the ss-gap regions, rendering the macrocycle mobile. Conversely, at 340 nm the nonplanar *cis*-AB forms, destabilizes duplex-formation by steric hindrance, and the release-ODNs dehybridize, rendering the ss-gaps in the ring and/or axle free to form the pseudostate of the respective interlocked structure. The principle of photoresponsive DNA hybridization by covalently tethering AB-moieties onto DNA strands was introduced by Hiroyuki Asanuma.[35]

DNA strand displacement by photoregulated ODNs is a useful advance of the "toehold" method. This technique employs partially hybridized DNA duplexes with overhangs to which a ssDNA complementary to the entire DNA can hybridize, leading to branch migration of the shorter strand. It has been widely employed in DNA nanostructures with dynamic properties, ranging from DNA machines like walkers,[36] spiders,[37] tweezers,[38,39] robot arms,[40] and other dynamic DNA nanostructures.[41,42] "Dynamic" denotes the directed reorganization of a DNA architecture in which both the geometry and the structure-determined functions are altered. The toehold itself serves as a recognition sequence, but also advances the structural transformation under thermodynamic control by pairing with a fully complementary counter strand.

Using light-controlled switching can overcome disadvantages of dilution and material consumption/waste generation of the toehold-approach. We sought to directly compare the toehold-based switching between a stalled pseudorotaxane and a fully mobile macrocycle[43] with an adequate test system.[28] In the toehold-approach we used the release-ODN th-RO that was complementary to the 12-mer ss region in the axle and contained a 7-nucleotide 5′-overhang. When th-RO was added to the pseudorotaxane, the hybridized macrocycle became released and mobile. Addition of cth, an ODN complementary to the entire

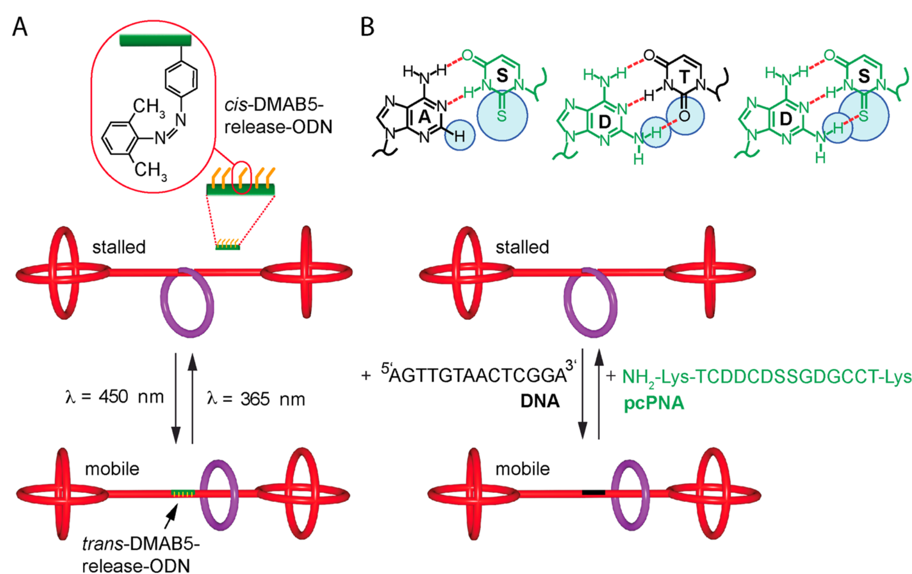

Figure 7. Switching of pseudorotaxane/rotaxane. (A) Light-dependent switching with a DMAB5-RO at different wavelengths.[43] (B) Switching by double-duplex invasion using pcPNA.[45] Adapted with permission from ref 43, Copyright 2012 American Chemical Society; and from ref 45, Copyright 2013 Oxford University Press.

th-RO, reverts the process. Alternate addition of th-RO and cth repeatedly switches between the two states.

After nonquantitative switching was observed for a 12-mer release-ODN containing six azobenzene moieties (AB6-RO), we synthesized Asanuma's more bulky 4-carboxamide-2′,6′-dimethylazobenzene (DMAB) phosphoramidite.[44] With this modification, we found quantitative and nonfatiguing switching behavior with a 12-mer release-ODN containing five DMAB modifications (DMAB5-RO; Figure 7A). The ability to operate functional DNA nanostructures by light in a noninvasive fashion that avoids repeated addition of ODNs to the system opens up exciting opportunities in dynamic DNA nanotechnology.

To expand the toolbox of switches for dynamic DNA nanotechnology, we explored alternative approaches that augment the spectrum of reversible switching mechanisms even further. Recently, we came up with a toehold-free switching process that relied on the so-called pseudocomplementary peptide nucleic acids (pcPNAs).[45] Peter Nielsen had shown that pcPNAs can undergo double-duplex invasion[46] due to the pc nucleobases. Pseudocomplementarity results from the incorporation of synthetic bases into a DNA, or a PNA, that tolerate the pairing with their respective natural counterparts, but not with each other. For example, replacing thymidine for 2-thiouridine (S) and adenine for 2,6-diaminopurine (D) prevents their pairing in a nucleic acid due to a steric clash between the sulfur in S and the 2-NH_2-group in D. However, the cross-pairing A-S and D-T, respectively, can occur unhindered. Based on this pairing behavior, pcDNAs can invade natural dsDNA at the end and lead to branching of the dsDNA. By double-duplex invasion, pcPNAs can also bind highly sequence-specifically to dsDNA, and the resulting pcPNA-DNA heteroduplex is thermodynamically more stable than the corresponding DNA−DNA homoduplex.[47]

Based on these considerations, we synthesized a modified dsDNA rotaxane in which the 8-mer ss-gap in the axle was made of pcDNA containing S and D instead of T and A, respectively, that hybridize to a complementary 8-mer ssDNA sequence in the 14-nt macrocycle gap (Figure 7B). Addition of a 14-mer ssDNA release-ODN displaced the macrocycle from the axle. The process that fixes the macrocycle back to the axle requires quantitative removal of the 14-mer release-ODN. A DNA sequence would preferentially bind to its complementary ss-gap region in the axle rather than detaching the release-ODN from the macrocycle. But the respective pcPNA prevents hybridization to the axle, due to its strongly invasive binding characteristics. Now the macrocycle hybridizes back to the axle to reorganize into the stalled pseudorotaxane. This switching cycle is fuelled by the formation of the thermodynamically more stable pcPNA-DNA hybrid duplex. This mode of switching is unprecedented in DNA nanotechnology. Because it differs mechanistically both from the toehold and the light-induced switch, it can potentially be combined orthogonally with these approaches, thus allowing distinct switches to be operated in parallel in DNA nanomachines that require sequential and reversible triggering of distinct events.

■ CATENANE AND CATENANE-LIKE NANOSTRUCTURES MADE OF CIRCULAR DNA

DNA catenanes can form in cells, and presumably play a role in chromosome condensation.[48] Compared to rotaxanes, catenated DNA nanostructures have been more widely used in structural and functional DNA nanotechnology, in most cases as ssDNA catenanes.[15] A dsDNA catenane was assembled by Alexander Heckel, who showed that the interlocked structure can be guided by equipping two DNA half-circles containing repetitive AT-tracts with Dervan-polyamides that bind the half-circles sequence specifically[49] so that their ends point into opposite directions. Held together by this supramolecular assembly, the two fragments were then ligated to a ring-closing ODN to produce the interlocked catenanes.

Using DNA origami, Hao Yan et al. assembled a Möbius strip, a topology that has a single surface and one boundary edge. By cutting along selected DNA helices, a strand-displacement can occur that reconfigures the Möbius strip into other topologies such as a catenane of two interlocked rings.[50]

Catenanes that were largely single-stranded were employed for the reversible and switchable programming of the positions of DNA strands, the location of which was read out by scanning

transmission electron microscopy (STEM) of attached Au-NPs.[51] Another study described a ss two-ring rotaxane that addressed three defined positions depending on different external input signals.[52] Different pH-values directed i-motif formation or disassembly, and Hg^{2+}/cysteine switched between Hg^{2+}-bridge-assisted base pairing and removal of Hg^{2+} by cysteine. By combining these triggers, directional addressing of the three "stations" of the "rotor-ring" was demonstrated by fluorescence, resembling a rotation-like movement of the rotor ring around the 'track-ring' (Figure 8).

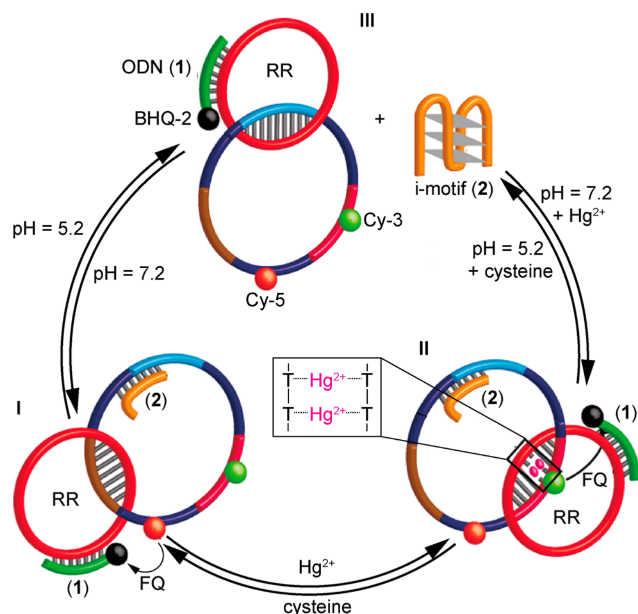

Figure 8. Reversible signal-triggered translocation of the rotor ring α across the three states I, II, and III (modified from reference 52).[52] RR, rotor ring; BHQ-2, Black-hole-quencher-2; FQ, fluorescence quenching. Adapted with permission from ref 52. Copyright 2013 American Chemical Society.

FUTURE PROSPECTS

Small dsDNA nanorings are highly versatile building blocks for structural and functional DNA nanotechnology, due to their shape persistence and preorganization resulting from DNA bending by repetitive AT-tracts. We have developed a straightforward, reliable, and modular threading method that provides access to an innovative class of interlocked dsDNA nanoobjects of circular geometry as versatile components for nanomechanics and nanorobotics. We have applied this method for generating dsDNA pseudorotaxane and rotaxane architectures. The modular nature and the step-by-step assembly will enable the straightforward construction of multiply interlocked, ds-rotaxane, ds-catenane, and other interlocked species that open up new avenues in synthetic biology and nanomachinery.

We can now employ these threading paradigms for the construction of DNA-, aptamer-, and ribozyme-hybrid architectures containing interlocked structures wherein individual components can be set in motion in a controlled manner for achieving a function. Several methods are available for switching and will be useful for creating autonomously working DNA nanomachines, which can perform mechanical work and put nanoscale components in motion. Toward that goal, naturally evolved biological systems provide highly complex, yet well-defined structural and functional units that could be combined with DNA nanostructures to serve as autonomously functioning building blocks in biohybrid nanomachinery.[5] The challenge now is to merge functional DNA building blocks with these biological components without loss of functionality.

Many interlocked supramolecular cognate motifs have been created by organic and inorganic chemistry to construct molecular architectures, which can be exploited to control submolecular motion by applying external stimuli. Many examples show that the stimulated movement of components can be used to vary all kinds of physical properties. Extending these approaches to well-defined, highly shape-persistent, and preorganized interlocked dsDNA nanostructures will open up an even larger arena of applications. Not only is DNA superior over other construction materials due to the predictable interactions that lead to ODN-hybridization. But compared to the usually much smaller interlocked molecules created from organic and inorganic chemistry, the larger DNA architectures described to date expand the size-range of interlocked molecules from a few nanometers to the submicrometer scale. This is an important step as it allows for a dramatic increase of the dynamic margins of nanomechanical devices. Moreover, it will increase the number and repertoire of functional or associative modules that can be incorporated into a single nanostructure because DNA can accommodate a huge variety of functional units, from proteins to RNA and PNA, from micelles and biological membranes to chemically synthesized entities. Altogether, mechanically interlocked dsDNA architectures provide highly promising functional units for DNA nanotechnology with an enormous innovative potential and implications ranging from chemistry to synthetic biology, and from the life sciences to nanoengineering.

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Notes

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