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A novel family of structurally stable double stranded DNA catenanes†

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Here we describe the design, assembly and characterisation of different structurally stable and highly polyvalent DNA catenanes. We synthesized a series of different catenated DNA nanostructures, among them symmetric ones containing two 126 or 168 base-pair rings, non-symmetric ones with a 126 and a 168 base-pair ring, and a [3]catenane containing three 126 base-pair rings. Reversible and quantitative on/off switching of the mobility of the rings was demonstrated as a proof-of-concept for the employment of these catenanes as dynamic DNA-nanostructures.

Interlocked molecular assemblies such as rotaxanes, catenanes, or knots have gained increased interest over the last two decades as they display controlled translational and rotational mobility between their individual components, which are held together through non-covalent mechanical bonds.[1] This enables their motion to be highly directional and repeatable and thus, these architectures have been widely used as scaffolds for the construction of functional nanomachines and devices such as molecular switches, muscles, shuttles or artificial machines and motors.[2]

DNA nanotechnology has explored mechanically interlocked architectures made of DNA to develop highly complex structural and functional assemblies such as Borromean rings,[3] knots,[4] or catenanes.[5] However, most of the macrocycles contained in these topologically interlocked structures are based on ssDNA. This poses certain limitations, particularly in cases where robust structural motifs with controlled mobility between their components are needed. Thus, the lack of rigidity and structural stability and the possible formation of unwanted secondary structures due to misfolding or undesired base pairing can hamper the use of largely single-stranded interlocked motifs in complex DNA-based nanomachinery. To address this drawback, we have previously reported structurally stable double-stranded DNA (dsDNA) nanorings,[6] the intrinsic curvature of which resulted from repetitive A/T-tracts. These dsDNA nanorings were employed for assembling different structures and interlocked functional motifs, such as dimeric and oligomeric aggregates,[7] catenaned structures assembled through Dervan-type polyamides[8] or RNA–RNA interactions,[7a] and rotaxanes.[9] Whereas the polyamide-containing catenane is trapped in a pseudo-catenane state under native conditions, the rotaxane shows an unhindered translation and rotation of the macrocycle along the axle. In the rotaxane system we implemented a light-sensitive on/off switch to control the mobility of the macrocycle.[9c] Other studies reported single-stranded DNA polycatenated structures able to switch between different states and conformations through several stimuli including pH changes, ions (Hg^{+2}) or using strand-displacement oligodeoxynucleotides (ODNs).[5,10]

Herein, we present the design, synthesis and characterization of a new class of dsDNA [2] and [3]catenanes. These nanostructures consist of two or more rigid and entirely dsDNA macrocycles of different sizes that self-assemble by following fundamental Watson–Crick base pairing rules.

Thus, in contrast to the previously reported polyamide-modified catenanes,[8] no chemical functionalization of the ODNs is required. Furthermore, the interlocked macrocycles can move unhindered relative to each other without any restriction. To obtain rigid macrocycles with predicted radii, the sequences were designed with distinct lengths and content of repetitive A/T-tracts. As described previously, these A/T-tracts lead to a curvature of the dsDNA[11] and therefore provide well-defined rings of even circular shape, in which neither bending nor contraction occurs. Also, as pointed out above, undesired secondary structures are prevented. This is especially important for functional systems in which two or more stable states need to be spatially separated.[12] In such systems, collapse or wrong arrangement of the structure can lead to a less effective or even non-functional system. Indeed, in a bienzymatic system using a rigid DNA origami as a scaffold it was observed that tuning the distance between the two enzymes resulted in a dramatic change in the overall activity.[13] Moreover, as previously reported, these dsDNA nanocircles can be easily functionalised with a wide variety of DNA structural motifs such as T-junctions,[7b] sticky-ends and even 4-way

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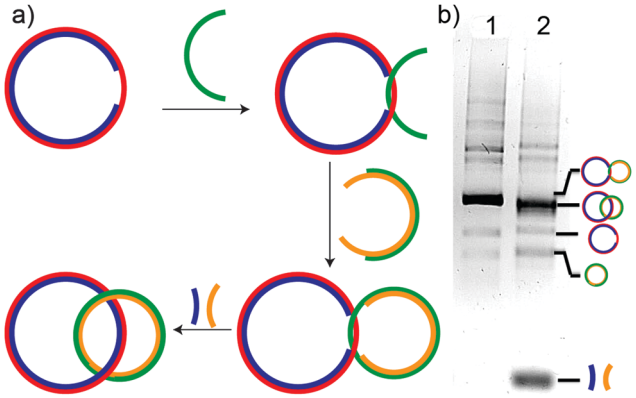

Fig. 1 (a) Schematic for a non-symmetrical [2]catenane assembly. (b) Analytical agarose gel of the assembled non-symmetric catenane. Lane 1, pseudocatenane, lane 2, catenane after addition of ROs.

Holliday-junctions.[9a] These features inherently increase the scope and versatility of the nano-assemblies into which these rings and catenanes can be incorporated.

The threading of the different rings and, consequently, the assembly into catenane structures is illustrated in Fig. 1a. First, a ring containing a single stranded gap region was synthesized. Then, an ODN containing a complementary sequence towards the gap region and two sticky-ends was added, allowing its hybridisation with the ring. Subsequently, the second ring was closed by adding its remaining pre-assembled part, designed to hybridize with the sticky-ends of the threaded ODN. To finally convert the assembled pseudocatenane into a catenane with mobile rings, release ODNs (ROs), entirely complementary to the gap regions of both rings, were added, resulting in two completely double stranded and interlocked DNA rings (for secondary structures and sequences see the ESI,† Fig. S1 and Table S1). To enhance the stability and resistance against certain nucleases, all nicks were enzymatically ligated.

Using this straightforward methodology, we assembled symmetric catenanes containing two 126 or 168 base-pair (bp) rings, non-symmetric catenanes with one 126 and one 168 bp ring, and a [3]catenane containing three 126 bp rings. The assembly of the catenanes was monitored by agarose gel electrophoresis (Fig. 2a) and the integrity of the catenated structures was corroborated by denaturing PAGE (Fig. S3, ESI†). The pseudocatenane/catenane conversion was monitored both by electrophoretic mobility studies and fluorescence experiments (Fig. 1b and Fig. S4, ESI†). Note that upon release of the two rings the catenane showed slightly faster mobility in agarose gel than its pseudocatenane analogue, similar to previous observations with other interlocked systems.[9a]

For possible future applications, our aim was to achieve a high degree of purity combined with a relatively high yield. Therefore, the purification of the nanostructure was performed using weak-anion-exchange HPLC. The HPLC chromatogram (Fig. S5, ESI†) shows that the catenated structure is predominantly formed.

To prove the feasibility of our system as a platform for complex applications, we sought to explore the robustness and rigidity of these assemblies, as compartmentalization and structural

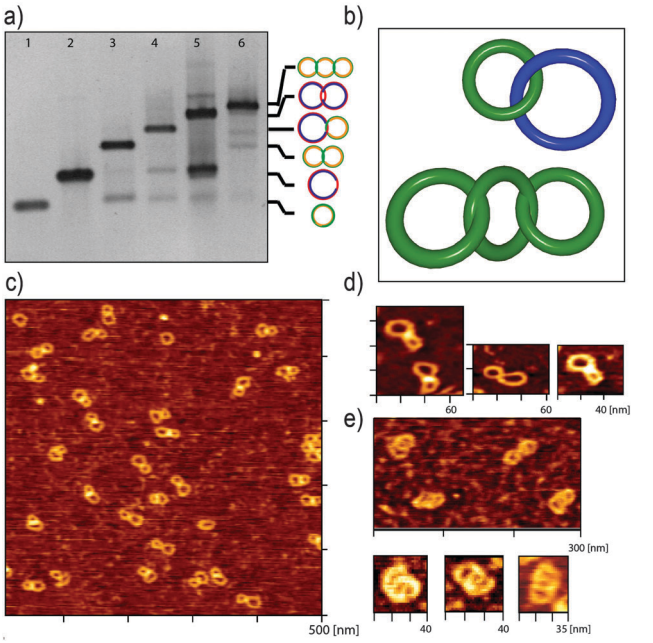

Fig. 2 (a) Analytical agarose gel of the 126 bp ring (lane 1), the 168 bp ring (lane 2) and the catenanes containing two 126 bp rings (lane 3), one 126 and one 168 bp ring (lane 4), two 168 bp rings (lane 5) and the [3]catenane consisting of three 126 bp rings (lane 6). (b) 3D representation of how the asymmetric catenane and the [3]catenane arrange on the modified cationic mica surface for AFM. (c) AFM image of the non-symmetric catenane. 0.5 μm^2 area measured in intermittent contact mode in air. (d) AFM images (tapping mode in liquid) of these catenanes. The radii of rings were in agreement with the calculated ones (13.6 and 18.1 nm for the 126 and the 168 base pair rings, respectively). (e) AFM images of the [3]pseudocatenane (intermittent contact mode in air).

differentiation are key requirements for their utilization in many functional devices.[14] Therefore, AFM studies of the non-symmetric [2]catenane were performed. In Fig. 2c it is shown that nearly all catenane structures remain intact after deposition on the surface. All catenanes exhibit a well-defined, smooth circular shape, as expected. Moreover, the two different macrocycles can be easily identified, and the radii found after cross section statistical analysis of the sample for each of the rings fit well with the theoretical ones (Fig. S6, ESI†). These results set forth a high degree of control on the nanoscale that we can achieve with these interlocked macrocycle structures. Ultrahigh-resolution AFM analysis at the solid/liquid interface in HyperDrive™ mode confirmed the regular shape of the catenated structures showing almost no deformation or contraction (Fig. 2d). The intrinsic robustness of these systems allows us to visualize the [3]catenane structure with sufficient resolution, by using regular AFM intermittent contact mode (Fig. 2e and Fig. S7, ESI†).

We next implemented a toehold-based switch into the symmetric catenane consisting of two 168 bp rings. Repetitive addition of a toehold-RO (TH-RO) and the corresponding complementary ODN (Comp-RO), respectively, switches the catenane reversibly between the immobile pseudocatenated state and the state in which the two rings can rotate and move unhindered with respect to each other (Fig. 3). The ability to precisely control the switching behaviour of the catenated DNA assembly is an important feature of these interlocked

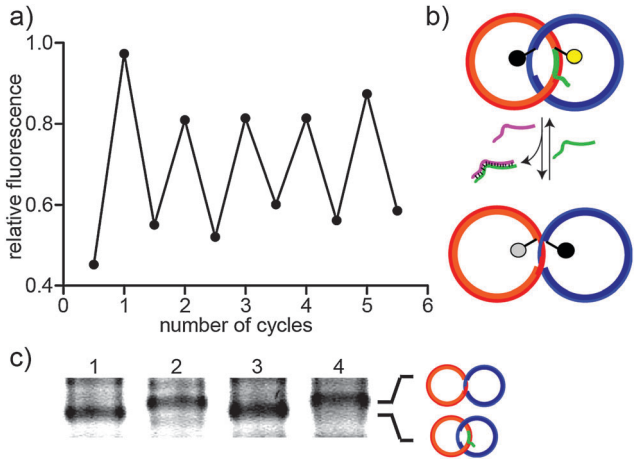

Fig. 3 (a) Changes in the fluorescence intensity due to reversible catenane/pseudocatenane conversion. (b) Schematic of the toehold mediated switch. Addition of the TH-RO (green) leads to dehybridization of the rings and therefore the mobile state. Addition of the Comp-RO (pink), totally complementary to TH-RO, reverts this process by forming the stable TH-RO/Comp-RO duplex. In the pseudocatenene state, the fluorescence is quenched due to close proximity between the fluorophore (Cy3, grey dot) and the quencher (BHQ-2, black dot). In the catenane state, dequenching of Cy3 (yellow dot) is observed. (c) Analytical agarose gel of the switching. Lanes 1 and 3 correspond to catenane, lanes 2 and 4 to pseudocatenane, respectively.

systems that allows implementing triggered function into complex dynamic DNA nanostructures. Labelling of the rings with a fluorophore–quencher pair allowed the detection of the pseudocatenaned or catenaned states, respectively, by fluorescence quenching. Fig. 3b illustrates the reversible switching mechanism, which shows low fatigue even after 5 complete switching cycles (Fig. 3a). An electrophoretic mobility study was performed to determine the switching efficiency. As evident from Fig. 3c, the conversion is nearly quantitative upon addition of the corresponding ODNs.

To conclude, we have shown the design, assembly and characterization by gel electrophoresis and AFM of a new family of double stranded DNA catenanes. The described interlocked structures can be synthesized in a straightforward way, by using commercially available ODNs, and isolated by weak anion exchange HPLC chromatography affording highly pure and homogeneous samples. Using DNA as basic material, we provide water soluble, highly modular, synthetically accessible and biocompatible interlocked catenanes assembled from dsDNA nanorings of different radii. Thus, in contrast to other reported synthetic organic and inorganic structures, our catenanes can in principle be decorated with proteins, DNA- and RNA-motifs like DNAzymes, ribozymes or kissing loops. The robustness, homogeneity and well-defined shape of these assemblies were unequivocally assessed using ultrahigh-resolution AFM (Hyperdrive™). By using Hyperdrive™ AFM, there is no need for external markers (like nanoparticles or fluorophores) to distinguish between the different rings and thus to unambiguously determine distinct conformations in such interlocked systems. In comparison with previously described largely ssDNA catenanes,[5] the catenanes introduced here provide alternative scaffolds suitable for incorporation into functional DNA nanostructures that require a more rigid spatial separation. Furthermore, we have shown the reversible and quantitative on/off switch of the mobility of the rings, as a proof-of-concept for the future employment of these catenanes as dynamic nanostructures. Overall, the here-described intrinsic features of these interlocked DNA assemblies pave the way for their use and implementation as structural motifs in complex chemical and biological nanosystems such as shuttles, artificial machines, and motors.

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