Assembly of dsDNA nanocircles into dimeric and oligomeric aggregates†

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The assembly of double-stranded (ds) DNA nanocircles both by hybridization with branched oligodeoxynucleotides (ODNs) and by intercalation was analyzed by atomic force microscopy (AFM). Branched ODNs ligated to single-stranded (ss) gap regions of dsDNA nanocircles led to defined, dumbbell-shaped architectures. ODNs containing an aromatic intercalator yielded oligomeric aggregates.

Nucleic acids are an ideal material for the construction of nanometre-scaled objects. DNA-based scaffolds of different topological and mechanical properties have been generated and applications for ordered nucleic acid nanoarchitectures have been implemented. Nucleic acids have also been used for building devices like walkers,[1] tweezers,[2] thermometers,[3] or self-replicating systems,[4–6] and for the directed conjugation of biomaterials. In programming the assembly of higher-order nanoparticle structures in which DNA is used as a mould, supramolecular principles that go beyond the classical Watson–Crick-based association of DNA-strands have been harnessed.[7] Among them, the hybridization of chemically functionalized ODNs with metal-chelating groups,[8,9] intercalators,[10] nanoparticles,[10–12] or externally added molecular struts[13] has proven useful.

We have previously described the efficient synthesis of dsDNA minicircles containing bespoke single-stranded (ss) gap regions.[14,15] These dsDNA-circles hardly exhibit any ring strain because they contain repetitive, intrinsically bent AT-tracts that cooperatively result in a circular shape of the double helix with 105, 126, 147, 168, or more base-pairs (bp).[16] The ss-gaps can be used as handles for functionalizing the dsDNA-rings.[14] We have established a new type of mixed DNA architectures, where RNA-aptamer motifs[15] or polypeptide struts[13] directed the assembly of circular dsDNA nanoobjects into higher order architectures in a precisely defined fashion. The ss-gap also allowed the introduction of chemical groups into a dsDNA nanoring. For example, we synthesized anthracene-modified ODNs containing one, two, or more anthracene residues in a single 21-mer ODN that is complementary to the gap.[14] Hybridizing these ODNs to the ss gap region provided access to dsDNA nanocircles containing anthracene modifications at defined positions. The anthracene groups were attached to C5 of deoxyuridyl residues *via* a flexible linker in a way that the intercalator protrudes from the helix by a length of approximately 10 Å (Fig. 1). This length is sufficient for the aromatic residue to intercalate into a neighbouring dsDNA nanocircle, but is short enough to prevent self-intercalation. Such a mode of interaction could be cooperative in a way that either results in a combination of nanocircles at their sites of functionalization, which would lead to the assembly of two nanocircles, or, alternatively, could be omnidirectional, leading to oligomeric aggregation of multiple nanocircles.

Here we have addressed this issue by analysing the self-assembly of anthracene-containing dsDNA nanocircles by atomic force microscopy (AFM). dsDNA nanocircles are well

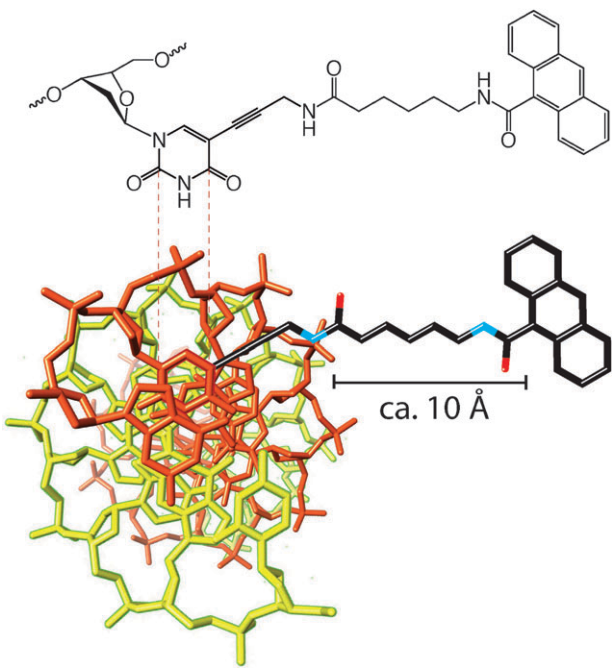

Fig. 1 Cross-section (view along the helix axis) of a molecular model of a dodecameric dsDNA (pdb: 1BNA) functionalized with a 5-[3-anthracene-9-carbamido)-ε-aminocaproylamido)-prop-1-inyl]-2′-deoxyuridyl residue (top).[17] The linker arm protrudes from the helix by about 10 Å, which is sufficient for the anthracene to intercalate into a neighbouring dsDNA.

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† Electronic supplementary information (ESI) available: AFM scan of additional dsDNA minicircles in the presence of the anthracene-modified ODN, AFM scan of ss-gap containing dsDNA minicircles (bottom panel) in the absence of the anthracene-modified ODN, Sequences of the gap-ring and strut ODNs. See DOI: 10.1039/c0cc00390e

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suited for this purpose as they exhibit sufficient dimensional stability to visualize their aggregation on surfaces, allowing insight into the mode of interaction of intercalator-functionalized dsDNA-nanocircles. We directly compare the intercalator-guided assembly with that obtained when using a branched ODN that can hybridize to the gap region of two nanocircles in a precisely defined fashion.

We applied a 21-mer ODN containing two anthracene moieties in position T6 and T16. A five-fold excess of the modified ODN (1.2 pmoles) was hybridized to a 168-mer dsDNA nanocircle containing a complementary 21-mer ss-gap sequence (0.23 pmoles) as previously described[14] (ESI† Fig. S1). The DNA was deposited from a buffered solution in the presence of 10 mM Ni^{2+} on a mica surface, and scanned by AFM in MAC-mode.[18] Whereas fully double-stranded nanocircles without anthracene-modification displayed single rings of uniform shape,[14] the scans obtained in the presence of the anthracene modified ODNs differed considerably (Fig. 2). Besides the sporadic occurrence of isolated circles and linear DNA fragments, all AFM scans revealed the frequent appearance of dimeric or oligomeric aggregation of DNA circles, sometimes also including linear ODN-fragments. Although the stoichiometry of some of these agglomerates varies, clusters of a defined number of two or three interaction-partners are clearly detectable (Fig. 2 and Fig. S2, ESI†).

We also performed an AFM analysis of the gap-containing ssDNA nanocircles in the absence of the anthracene-modified ODN under otherwise identical conditions. Although this analysis exhibited the sporadic presence of DNA-aggregates of not more than two nanocircles, the overall density of aggregates was clearly less pronounced as compared to the situation in the presence of the intercalator, and the majority of nanocircles appeared in single, non-aggregated form (ESI† Fig. S3).

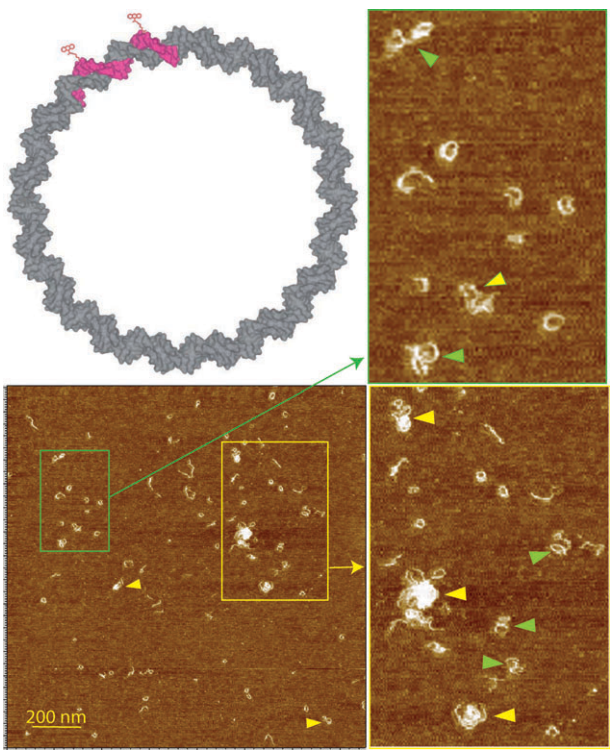

Fig. 2 Atomic force microscopy (AFM) scans in MAC-mode of 168-bp dsDNA nanocircles hybridized with a complementary anthracene-modified ODN (shown in magenta in the upper left panel). Zoomed sections (yellow and green frames) show aggregates of two (green arrows), or more (yellow arrows) dsDNA nanocircles.

These data are in accordance with the interpretation that aggregation of dsDNA nanocircles is mediated by sequence-independent intercalation of the anthracene groups. This interaction leads to pairs or oligomers of dsDNA-rings, and sometimes also to the linkage between linear dsDNA–dsODN fragments and dsDNA nanocircles. The interaction of an anthracene-modified DNA-ring with another functionalized ring does not prevent further aggregation of additional species. In the absence of intercalator the association of these architectures occurs at a considerably reduced level. As expected, the forces between the intercalator-functionalized DNA-rings are apparently weak; although ring assemblies can be visualized on mica surfaces, our attempts to detect them by other methods such as gel-shift experiments or plasmon resonance were not successful (data not shown).

Having verified the frequent occurrence of dimer- and oligomeric aggregates of anthracene-modified dsDNA nanocircles and its correlation with the ability to intercalate, we next sought to compare this non-directional mode of interaction with a precisely defined association system. For that purpose, we constructed a simple branched ODN that consisted of a linear dsDNA of 29 bp containing 10-mer ss overhangs on each side for hybridization with the ss-gap in the gap ring (Fig. 3a, for sequences see ESI†, Fig. S4). When hybridizing to a single gap-ring, a completely double-stranded DNA-nanocircle with a protruding arm should form, whereas

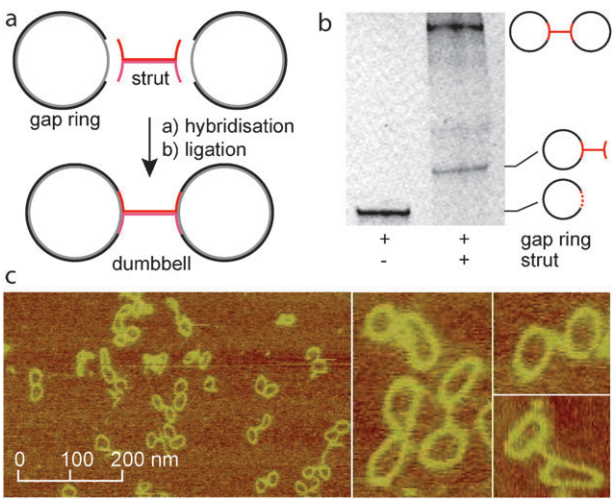

Fig. 3 A double-stranded dumbbell DNA topology. (a) Schematic for the dumbbell-topology that results from hybridization and ligation of the branched ODN-strut (red) to the ss-gaps of two DNA nanorings. (b) Polyacrylamide gel electrophoresis separates monomeric and dimeric associates of the strut-ODN with one or two DNA nanorings. (c) Atomic force microscopy (AFM) scans in MAC-mode of 168-bp dsDNA nanocircles hybridized and ligated to the complementary branched ODN-strut (left panel). Zoomed sections (right panels) display the well-defined and precise association of two dsDNA nanorings to form a dumbbell topology. In some of these topologies, the connecting linear dsDNA of 29 bp is unambiguously resolved.

hybridization to two rings should result in a dumbbell-shaped topology. Indeed, PAGE analysis confirmed that these topologies assemble in precisely defined stoichiometry, and can be ligated together using DNA ligase to yield a genuine dumbbell architecture (Fig. 3b).

To confirm the dumbbell topology an AFM analysis of the purified ligation product was carried out. Fig. 3c shows the uniformly dimeric structure of these DNA-architectures. Although the connecting linear strut-ODN is clearly visible in some of the structures, most of them exhibit similarity with the AFM-scans of the dimeric associates obtained by intercalation (Fig. 2 and Fig. S3, ESI†). As previously observed for single, entirely double stranded 168-bp DNA-nanocircles,[14] the flanking rings in the dumbbell architectures do not exhibit any significant ring deformation but rather display a uniformly circular structure. The highly straightforward way of generating these dsDNA dumbbell topologies is of relevance for the construction of more complex DNA architectures based on circular geometries. Indeed, we have recently developed a straightforward, reliable, and modular threading method that provides access to a previously unknown class of interlocked dsDNA nanoobjects of circular geometry as versatile components for nanomechanics and nanorobotics.[19]

In summary, we have shown that two aromatic intercalator moieties attached to a 168-bp dsDNA nanocircle are capable of forming dimeric or multimeric DNA ring aggregates. The unspecific adhesion forces based on intercalation are too weak to detect higher ring aggregates on gel but AFM analyses confirmed the existence of an unspecific assembly of DNA rings, demonstrating the potential of intercalation as a principle to assemble higher ordered DNA architectures from smaller subunits. Assemblies of two, three, or more DNA-rings, and stoichiometrically undefined clusters of nanorings and linear ODNs could be visualized by AFM. In contrast, precisely defined, uniform nanoarchitectures were obtained in the presence of a two-armed branched ODN that was used as a molecular strut to direct the assembly of two DNA-nanocircles by hybridizing to their ss-gap sequence. The hybridized strut/ring conjugates could be covalently manifested by ligation to yield an entirely double-stranded sub-type of DNA-nanoarchitecture in which two dsDNA-nanocircles are connected by a 29-mer linear DNA through three-way junctions. The resulting dumbbell-shaped topology was unequivocally confirmed by AFM experiments. The direct comparison of the sequence-independent, intercalation-directed assembly of DNA-nanoarchitectures with the use of DNA struts allows an assessment of these approaches for further exploration in DNA nanotechnology. Certainly, the use of sequence-specific molecular struts is a simple and highly versatile approach for the fast and specific assembly of smaller DNA subunits into geometrically defined, complex DNA architectures. Furthermore, these examples show that dsDNA nanocircles increase the versatility of nucleic acids as a construction material on the nanometre scale.

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