DNA Structures (1)

DOI: 10.1002/anie.200704004

DNA Minicircles with Gaps for Versatile Functionalization**

*Goran Rasched, Damian Ackermann, Thorsten L. Schmidt, Peter Broekmann, Alexander Heckel, and Michael Famulok**

The programmable self-association of molecular units into higher ordered structures plays a key role in the bottom-up construction of nanomaterials.[1] Crucial for the successful supramolecular assembly of nanoobjects, however, is the choice of the functional molecular units themselves. Nucleic acids have emerged as a convenient target for these purposes because of their unique properties in molecular recognition and the ease by which oligonucleotides can be accessed synthetically.[2,3] The assembly of DNA nanoobjects with defined two- or three-dimensional geometries requires either rigid subunits, such as in double crossover (DX) or paranemic crossover (PX) elements,[3] or inherently rigid triangular shapes such as tetrahedra or bipyramids.[4]

From a structural point of view, DNA minicircles are probably the simplest rigid objects with a nanometer size. Small DNA circles were first prepared by designing two 21-mer DNA precursor sequences which, upon hybridization and ligation, resulted in a statistical distribution of DNA minicircles containing 105, 126, 147, and 168 base pairs (bp).[5a] Atomic force microscopy (AFM) analysis of 168-bp minicircles confirmed their smooth circular structure without any ring deformation or supercoiling.[6,7] These features predestine them as building blocks for the assembly of objects on the nanometer scale. However, a major drawback of DNA minicircles in the construction of higher ordered DNA architectures is their unbranched, continuously double-stranded (ds) nature that prevents the guided aggregation of multiple rings. Furthermore, the statistical assembly of the short oligonucleotides that were applied in the known strategies for the synthesis of DNA minicircles[5–7] prevents the controlled introduction of "customized" sequences into the circle that can serve as defined handles for the self-assembly of multiple rings.

We recently reported the assembly of two DNA minicircles, guided by a "strut" of Dervan-type polyamides that specifically held two rings together by binding to different 9-mer double-stranded "custom" sequences that were present in each 168-mer ring exactly once.[8] Here we report on the construction of minicircles that contain a customized single-stranded gap sequence at a defined position. The gap can serve as a versatile site for the modification of DNA minicircles, thereby allowing, for example, their guided modification with functional groups through hybridization with synthetic oligonucleotides.

The key step in the preparation of DNA minicircles with sequence-specific functionalization focuses on a preformed incomplete minicircle MC_{gap} containing a 21-nucleotide single-stranded (ss) gap region (Figure 1). Hybridizing and ligating this gap-containing minicircle with any complementary 21-mer oligonucleotide ON_{funct} can lead to a variety of differently functionalized DNA minicircles MC_{funct} in a highly straightforward manner.

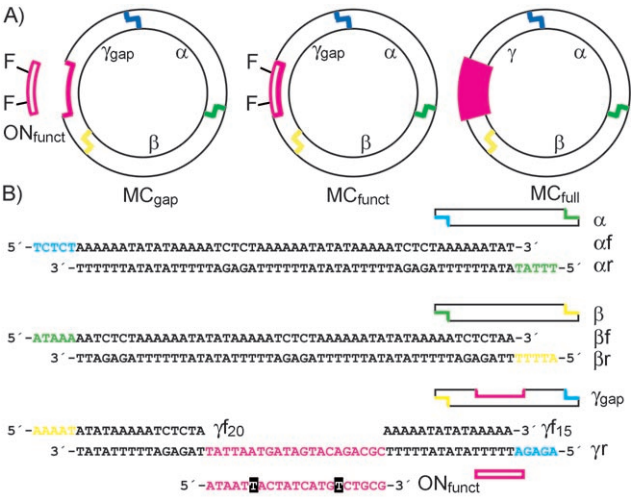

Figure 1. Design of gap-containing DNA minicircles. A) MC_{gap} (left): DNA minicircle with a single-stranded gap region for sequence-specific hybridization with a complementary functionalized (F) oligonucleotide (ON_{funct}). MC_{funct} (middle): functionalized DNA minicircle afforded by either hybridization or direct ligation with an ON_{funct}. MC_{full} (right): completely double-stranded DNA minicircle with a 21-bp custom sequence (red). B) Forward (f) and reverse (r) sequences of the dsDNA segments (α, β, γ) with individual 5′ overhangs (color coded) ensuring the assembly of 168-bp rings or multiples thereof. The red-colored 21-bp region in segment γ contains the customized sequence. Modified bases in ON_{funct} are framed in black.

[*] G. Rasched, Dr. D. Ackermann, T. L. Schmidt, Dr. A. Heckel, Prof. Dr. M. Famulok
Universität Bonn, LIMES-Life and Medical Science
Gerhard-Domagk-Strasse 1, 53121 Bonn (Germany)
Fax: (+49) 228-73-4809
E-mail: m.famulok@uni-bonn.de
Dr. P. Broekmann
Universität Bonn
Institut für Physikalische und Theoretische Chemie
Wegelerstrasse 12, 53115 Bonn (Germany)

[**] This work was funded by grants to M.F. from the Sonderforschungsbereich 624 (From the Design of Chemical Templates Towards Reaction Control). We gratefully acknowledge help from H. Rasched in generating the computer model of the minicircles.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

The prerequisite in the design of DNA minicircles is the repetition of A-tracts in the forward strands (αf, βf, and γf; Figure 1) since they cause a bending of the helical axis.[9] To obtain circularly bent DNA double strands, cooperative bending of all A-tracts is required. This only occurs if $d(A)_5$ and $d(A)_6$ A-tracts are arranged in tandem repeats alternating with $d(N)_5$ random base pairs.[5] However, for our purposes, the synthesis of minicircles starting from two 21-mer precursor DNA strands is not suitable since the incorporation of a gap region is not possible.

To achieve this, we constructed 168-bp DNA circles from three segments α, β, and γ (Figure 1). All segments consist of a DNA double strand of 51 bp having five base overhangs on their 5′ ends (Figure 1B). The choice of the sticky ends is the crucial specifying factor to selectively form the 168-bp DNA circles and no other sized rings. Thus, the segments are designed so that they hybridize only in the defined cyclic order (α→β→γ→α). In addition, the 21-mer gap no longer follows the A-tract arrangement, since the incorporation of any custom sequence is desired.

After performing the ligation steps (Supporting Information, Figure S2), the DNA minicircles were analyzed and purified by native two-dimensional polyacrylamide gel electrophoresis (2D PAGE).[10] In the first dimension, the products were separated according to their size (Figure 2A). Lane 3 shows the crude ligation product of the DNA minicircle MC_{full}. Besides the product (band 4), the monomers (band 1) and dimers (band 2) are still present in the mixture, probably because of incomplete phosphorylation. To separate c-shaped (open-ring) DNA side products from closed DNA minicircles, lane 3 was excised completely and subjected to PAGE in the second dimension in the presence of chloroquine diphosphate.[10] As shown in Figure 2B, only band 4 splits up. Figure 2C supports the open-chain nature of the faster migrating spot, which is sensitive to digestion with nuclease Bal31, whereas the slower one represents the desired 168-bp DNA minicircle. This result shows that 168-bp DNA minicircles tolerate a 21-bp segment that lacks any A-tract arrangement. Interestingly, the formation of the gap-containing DNA minicircle MC_{gap} was also possible under these conditions, although we observed a slight increase in the amount of side products compared to the formation of MC_{full} (data not shown), presumably because the short precursor sequences γf20 and γf15 only form weak duplexes. All the ligation experiments thus yielded a distinct band, either representing MC_{full} or the so far unknown gap-containing minicircle MC_{gap}.

To further confirm the circular nature of the minicircles, MC_{full} and MC_{gap} were visualized by AFM. Figure 3A shows

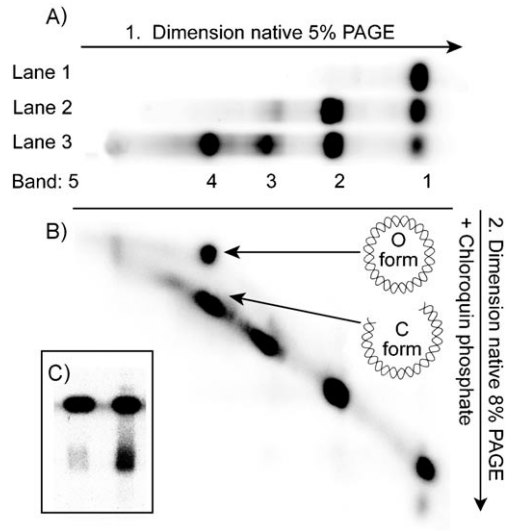

Figure 2. Identification of 168-mer DNA minicircles by 2D PAGE. A) The one-dimensional gel analysis separates the ligation products on the basis of the length of their base-pair segments. Lane 1: segment α; lane 2: products of the ligation of segments α + β; lane 3: products of the ligation of α, β, and γ. Band 1: one segment, monomer product; band 2: ligation product of segment dimers; band 3: unknown side product or secondary structure; band 4: trimer ligation products (168 bp). B) Two-dimensional gel analysis of lane 3 separates the circular (O-form) from the bent open (C-form) DNA. C) Two samples of the band 4 product (lane 3, gel (A) separated in a second dimension in the presence (left) and absence (right) of nuclease Bal31.

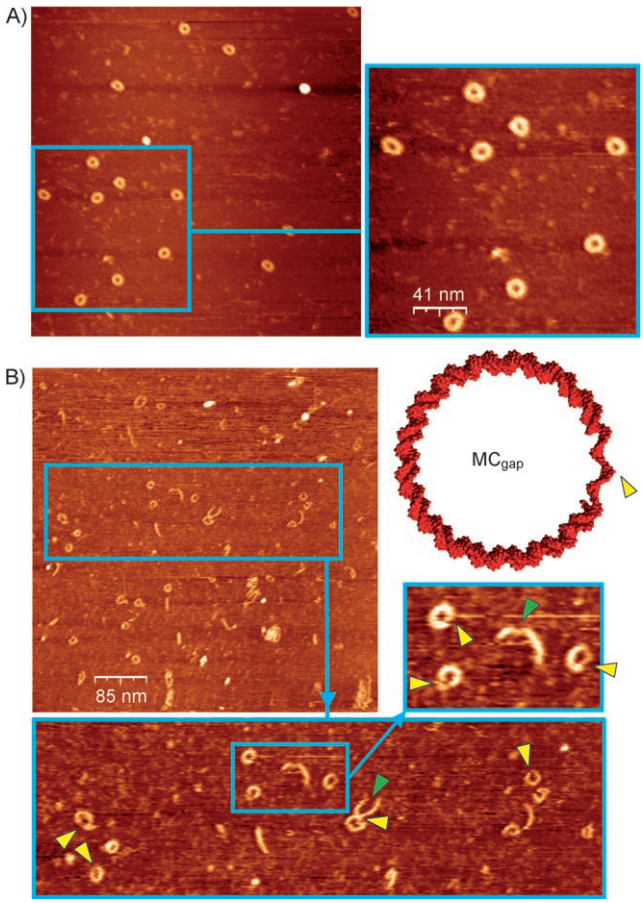

Figure 3. In situ atomic force microscopy (AFM) scans of DNA minicircles. A) MAC mode[11] AFM scan of DNA minicircles MC_{full} adsorbed on mica surfaces in the presence of 10 mM $NiCl_2$. The fully double-stranded DNA minicircles containing the custom sequence show no distortion and are of uniform shape and size. B) The same experiment with gap-containing DNA minicircles (MC_{gap}). Zoomed sections show DNA minicircles with a narrow region (yellow arrows). The model of a MC_{gap} (top right) shows the dimensions of the single-stranded region in relation to the ring. Green arrows: linear bent DNA fragments.

the uniformly circular structure of the fully double-stranded MC_{full}. Although these rings bear a 21-mer custom sequence lacking poly-A tracts, they do not show any ring deformation. The diameter of the rings is roughly 20 nm, which matches that of 18.2 nm expected for DNA minicircles with 168 bp (Supporting Information).

In contrast, the topology of the gap-containing minicircles MC_{gap} markedly differs from that of MC_{full} (Figure 3B). Clearly, these rings exhibit a higher degree of distortion, presumably because of an increased flexibility of the 21-mer single-stranded region, compared to the MC_{full} rings. Interestingly, all the circles show a single constriction along the outline of the ring, likely resulting from the single-stranded region, a phenomenon that is never observed for MC_{full}. While this topology dominates in the MC_{gap} scan, some linear bent shapes are also present, either because of damage induced by mechanical stress by the cantilever tip or exposure to shear forces during sample preparation and purification. Similar effects have been described previously in dsDNA scans.[7,11,12]

Having confirmed the circular structure of MC_{gap} by AFM, we next sought to equip MC_{gap} with additional chemical functionality by hybridizing it to a chemically functionalized DNA fragment. As an initial proof-of-principle, we designed a 21-mer oligonucleotide that was complementary to the gap sequence and contained two anthracene moieties at defined positions, attached to the C5-position of deoxyuridine residues through a spacer (Figure 4). The anthracene and the nucleobase were chosen for synthetic convenience and because these substituents point outside the double helix without affecting the DNA structure.[13] Furthermore, a slight stabilization of the DNA douplex is observed when the aminopropargyl residues of the spacer moiety are attached to pyrimidine nucleobases.[14] These considerations, together with the fact that anthracene residues can potentially intercalate into dsDNA, led to the use of ON_{funct} (Figure 1B) as the pilot oligonucleotide for the functionalization of DNA minicircles.

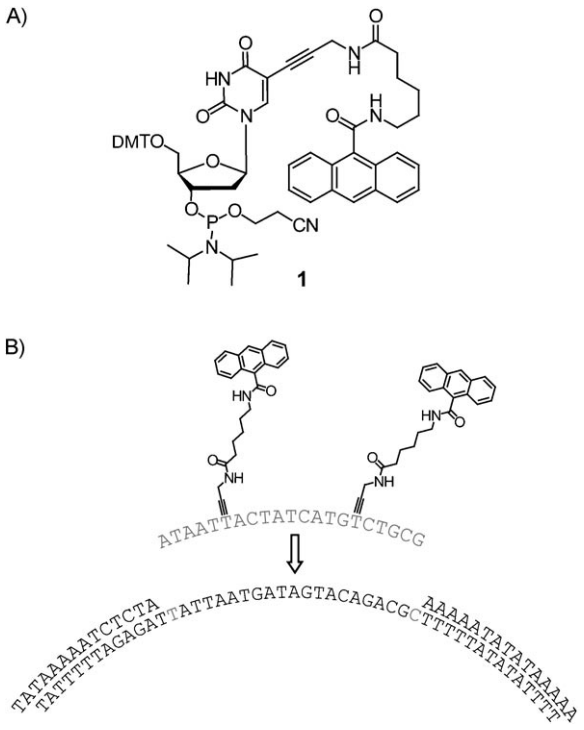

Figure 4. Anthracene-functionalized monomer and ON_{funct}. A) Phosphoramidite (**1**); DMT = 4,4′-dimethoxytriphenylmethyl. B) The functionalized oligonucleotide ON_{funct} hybridizes with its complementary region in the gap-containing minicircle MC_{gap}.

For the synthesis of phosphoramidite **1** (Figure 4A), we first derivatized the C5-position by a Sonogashira coupling reaction between 5-iododeoxyuridine and *N*-prop-2-ynyltrifluoroacetamide (Supporting Information, Scheme 1).[13] After changing the protecting groups, anthracene-9-carbamido-ε-aminocapronic acid was attached to the amino group using *N*,*N*,*N*′,*N*′-tetramethyl-*O*-(*N*-succinimidyl)uronium tetrafluoroborate (TSTU) as a coupling reagent.[15] The obtained functionalized nucleoside was converted into the corresponding phosphoramidite **1** under standard conditions.

ON_{funct} was prepared by automated solid-phase DNA synthesis. Phosphoramidite **1** was successfully incorporated into the 21-mer oligonucleotide at positions 6 and 16, with coupling efficiencies between 96–98%, as determined by analysis of the amount of trityl. After cleavage of the product from the solid support and removal of all base-labile protecting groups, ON_{funct} was purified by reverse-phase HPLC. ESI-MS studies on ON_{funct} confirmed the successful incorporation of two anthracene-modified deoxyuridine units.

We then hybridized ON_{funct} with the DNA minicircle MC_{gap}, thereby resulting in the highly sequence specific functionalized DNA minicircle MC_{funct} (Figure 4B). The relative gel mobilities of the three purified DNA minicircles MC_{full}, MC_{gap}, and MC_{funct} were examined (Figure 5). All three

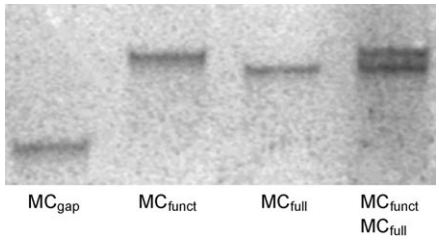

Figure 5. Analysis of the different DNA minicircles MC_{gap}, MC_{funct}, and MC_{full} by native PAGE.

circles showed the characteristic slow migration behavior known for DNA minicircles.[5] As expected, MC_{gap} has a higher mobility than the fully hybridized MC_{full} and MC_{funct}. Although these latter two species have the same charge and the same nucleobase sequence, they separate on the gel as a result of the presence of the two anthracene residues in MC_{funct}.

In summary, we have established a straightforward method for preparing highly sequence specific functionalized DNA minicircles based on chemical synthesis, the programmed self-assembly of DNA oligonucleotides, and enzymatic ligation. Crucial for that is the design of the precursor sequences in the cyclic order (α→β→γ→α), which not only

afforded completely dsDNA minicircles (MC_{full}) but also a gap-containing one (MC_{gap}), thus providing versatile intermediates to access functionalized DNA minicircles. Gap-containing minicircles can, in principle, be hybridized with any desired functionalized oligonucleotide to yield DNA minicircles with specific properties. The site-specific modification of DNA minicircles opens up new avenues for accessing higher ordered DNA objects based on building blocks with circular geometry.

Received: August 30, 2007
Published online: December 3, 2007

Keywords: DNA · DNA structures · functionalized DNA · nanostructures · scanning probe microscopy

[1] G. M. Whitesides, J. P. Mathias, C. T. Seto, *Science* **1991**, *254*, 1312.
[2] a) J. Wengel, *Org. Biomol. Chem.* **2004**, *2*, 277; b) N. C. Seeman, *Trends Biotechnol.* **1999**, *17*, 437; c) C. M. Niemeyer, *Angew. Chem.* **2001**, *113*, 4254; *Angew. Chem. Int. Ed.* **2001**, *40*, 4128; d) N. C. Seeman, *Nature* **2003**, *421*, 427.
[3] E. Winfree, F. Liu, L. A. Wenzler, N. C. Seeman, *Nature* **1998**, *394*, 539.
[4] a) R. P. Goodman, I. A. Schaap, C. F. Tardin, C. M. Erben, R. M. Berry, C. F. Schmidt, A. J. Turberfield, *Science* **2005**, *310*, 1661; b) C. M. Erben, R. P. Goodman, A. J. Turberfield, *J. Am. Chem. Soc.* **2007**, *129*, 6992.
[5] a) L. Ulanovsky, M. Bodner, E. N. Trifonov, M. Choder, *Proc. Natl. Acad. Sci. USA* **1986**, *83*, 862; b) Y. Lyubchenko, L. Shlyakhtenko, B. Chernov, R. E. Harrington, *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 5331.
[6] W. Han, M. Dlakic, Y. J. Zhu, S. M. Lindsay, R. E. Harrington, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 10565.
[7] W. Han, S. M. Lindsay, M. Dlakic, R. E. Harrington, *Nature* **1997**, *386*, 563.
[8] T. L. Schmidt, C. K. Nandi, G. Rasched, P. P. Parui, B. Brutschy, M. Famulok, A. Heckel, *Angew. Chem.* **2007**, *119*, 4460; *Angew. Chem. Int. Ed.* **2007**, *46*, 4382.
[9] a) A. Maki, F. E. Brownewell, D. Liu, E. T. Kool, *Nucl. Acids Res.* **2003**, *21*, 1059–1066; b) A. S. Maki, T. Kim, E. T. Kool, *Biochemistry* **2004**, *43*, 1102–1110; c) D. Crothers, T. E. Haran, J. G. Nadeau, *J. Biol. Chem.* **1990**, *265*, 7093.
[10] M. Dlakic, R. E. Harrington, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 3847.
[11] W. Han, S. M. Lindsay, T. Jing, *Appl. Phys. Lett.* **1996**, *69*, 4111.
[12] H. G. Hansma, M. Bezanilla, F. Zenhausern, M. Adrian, R. L. Sinsheimer, *Nucleic Acids Res.* **1993**, *21*, 505.
[13] a) S. Jäger, G. Rasched, H. Kornreich-Leshem, M. Engeser, O. Thum, M. Famulok, *J. Am. Chem. Soc.* **2005**, *127*, 15071; b) O. Thum, S. Jäger, M. Famulok, *Angew. Chem.* **2001**, *113*, 4112; *Angew. Chem. Int. Ed.* **2001**, *40*, 3990; c) S. Jäger, M. Famulok, *Angew. Chem.* **2004**, *116*, 3399; *Angew. Chem. Int. Ed.* **2004**, *43*, 3337; d) W. Frank, J. Hobbs, *J. Org. Chem.* **1989**, *54*, 3420.
[14] M. Ahmadian, P. Zhang, D. E. Bergstrom, *Nucleic Acids Res.* **1998**, *26*, 3127.
[15] a) W. Bannwarth, D. Schmidt, R. L. Stallard, C. Hornung, R. Knorr, F. Mueller, *Helv. Chim. Acta* **1988**, *71*, 2085; b) W. Bannwarth, R. Knorr, *Tetrahedron Lett.* **1991**, *32*, 1157.

