Supplementary Information

Assembly of dsDNA nanocircles into dimeric and oligomeric aggregates

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Instrumentation. Automated solid-phase oligonucleotide synthesis was performed on an ABI 394 DNA-Synthesizer (*Applied Biosystems*). Mass spectra of the oligonucleotides were performed on a *Bruker* APEX IV Fourier-Transform Ion-Cyclotron-Resonance (FT-ICR). Polyacrylamide gel electrophoresis (PAGE) was performed on a Mini Protean 3 system from *BioRad* and for the electroelution of the DNA architectures a 422 Electroeluter from *BioRad* was used. Instruments for measuring atomic force microscopy (AFM) see below.

Buffer solutions. TEAAc: 100 mM triethylammonium acetate in H_2O; DNA storage buffer: 50 mM NaCl, 10 mM Tris·HCl in H_2O, pH 7.5; TAE-Mg buffer: 40 mM Tris, 20 mM acetic acid, 2.5 mM EDTA, 12.5 mM $MgCl_2$, pH 7.5.

Synthesis of anthracene modified oligonucleotide. The anthracene modified oligonucleotide was prepared by standard phosphoramidite chemistry (0.2 µmol scale) and deprotected and cleaved from solid support by treating it with 1 mL aq. NH_3-solution (33%, DNA-grade) at 55°C for 5 h. The oligonucleotide was purified by RP-HPLC (Agilent 1100 HPLC System, Nucleosil 100-5 C18 column, CS-Chromatographie Service GmbH, Germany) Flow rate: 1.0 mL/min; buffer A: 0.1 M TEAAc in H_2O; buffer B: MeCN; gradient: 0 → 30% B in 20 min. The oligonucleotide was characterized by ESI-MS: calculated 7104.4, found 7127.7 [M+Na].

Synthesis of gap containing DNA nanocircles. All ssDNA were ordered as 5'-phosphate oligonucleotides from *Metabion*. For the ligation of the subunits the following optimised protocol was used: 4µM of each precursors ssDNA, 3'000 U/ml T4 DNA ligase (*New England Biolabs*) and 1x ligation buffer (*New England Biolabs*) in a total volume of 200µl. Prior to the addition of the ligase (400 u) the solution was annealed to 65°C and then allowed to cool down to 15°C during 3h. Ligation was performed at 15°C during 14 h. Complete consumption of the precursor DNA sequences was verified by native PAGE (7% acrylamide, 200 V, 40 min). As a reference a fully double stranded DNA nanoring was simultaneously run on the gel, indicating the correct band for the gap-containing DNA nanoring. The product band was excised from the gel and subjected to electroelution at 200V for 2h. After collecting the product solution the DNA nanocircles were isolated by EtOH precipitation and the pellet dissolved in 100 µl 1x DNA buffer.

Oligonucleotides for intercalator modified DNA nanocircle

name	sequence
AlphaI-f	5'-TCTCTAAAAAATATATAAAAATCTCTAAAAAATATATAAAAATCTCTAAAAAATAT-3'
AlphaI-r	3'-TTTTTTATATATTTTTAGAGATTTTTTATATATTTTTAGAGATTTTTTATATATTT-5'

BetaI-f	5′-ATTTTTTAGAGATTTTTATATATTTTTTAGAGATTTTTATATATTTTTTAGAGATT-3′
BetaI-r	3′-TTAGAGATTTTTTATATATTTTTAGAGATTTTTTATATATTTTTAGAGATTTTTTA-5′
GammaI-f	5′-AAAATATATAAAAATCTCTAATAATTACTATCATGTCTGCGAAAAATATATAAAAA-3′
GammaII-r	3′-TATATTTTTAGAGATTATTAATGATAGTACAGACGCTTTTTATATATTTTTAGAGA-5′
Delta1-f / Delta2-f	5′-AAAATATATAAAAATCTCTA-3′ 5′-AAAAATATATAAAAA-3′
Delta-r	3′-TATATTTTTAGAGATTATTAATGATAGTACAGACGCTTTTTATATATTTTTAGAGA-5′

Oligonucleotides for dumbbell-shaped DNA nanoarchitecture

name	sequence
AlphaII-f	5′-AGAAAAAAGTGCCAAAAAGACGGAAAAAACGCGCAAAAAGCGCGAAAAAAGGG-3′
AlphaII-r	3′-CGGTTTTTCTGCCTTTTTTGCGCGTTTTTCGCGCTTTTTTCCCGGTTTTTGAC-5′
BetaII-f	5′-CCAAAAACTGTCAAAAAACGGAGAAAAATGTTCAAAAAATATGGAAAAACGC-3′
BetaII-r	3′-AGTTTTTTGCCTCTTTTTACAAGTTTTTTATACCTTTTTGCGCGTTTTTTCT-5′
GammaII-f	5′-GCAAAAAAGACGGAAAAACTGTCAAAAAATGTTCAAAAAGCG-3′
Circle1-r	3′-GCCTTTTTGACAGTTTTTTACAAGTTTTTCGCCATTAATGATAGTACAGACGCTCTTTTTTCA-5′
Strut-f	5′-GTAATTACTAAGGAACTCTCGCCAATAGGCATTGCTATTTCATGTCTGCG-3′
Strut-r	3′-GCGTCTGTACTTCCTTGAGAGCGGTTATCCGTAACGATAAATCATTAATG-3′

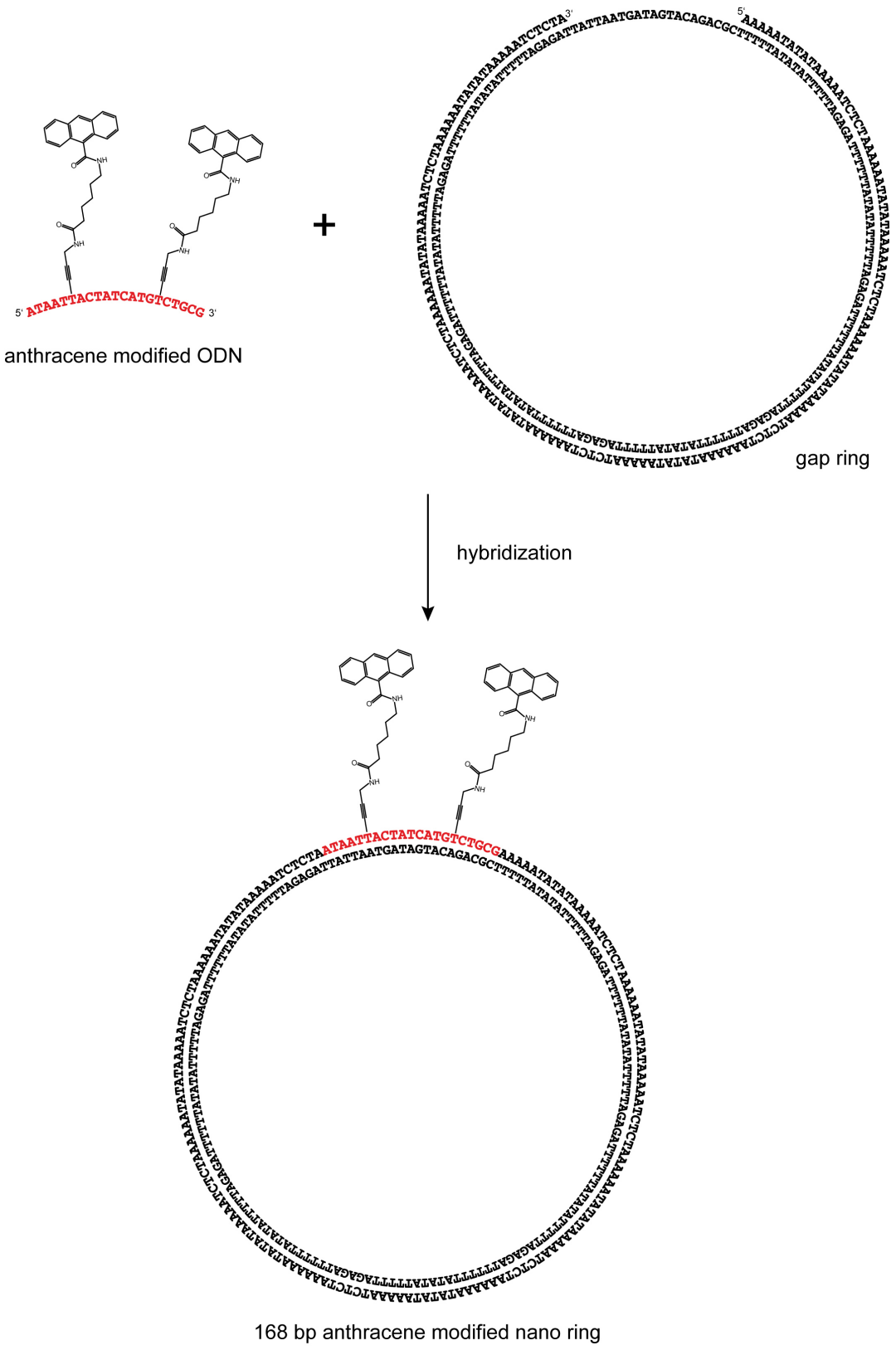
Preparation of anthracene functionalized DNA nanocircles. The anthracene modified ODN (5 equiv.) was annealed to the DNA gap ring (1 equiv.) in 1x DNA storage buffer. This solution (~ 5 nM) was directly used for adsorption of the circles on mica surface.

Preparation of dumbbell-shaped DNA architecture. The two DNA strut sequences (2.5 pmol, 1.0 equiv. Strut-f, 1.0 equiv. Strut-r) were annealed in ligase buffer and the gap-containing DNA nanocircles (5.0 pmol, 2.0 equiv.) added at 15° C in a total volume of 25 µl. The sample was treated with ligase (80 u) for 5h at 15° C. The crude was separated using PAGE (6% acrylamide, 200 V, 40 min) followed by electroelution of the product band and EtOH precipitation.

AFM imaging methode I (used for gap containing and intercalator-modified DNA nanorings).
Atomic force microscopy (AFM) was conducted in a liquid cell with a PicoScan AFM (former Molecular Imaging Inc. now Agilent Series 4500 SPM, PicoSPM I) using magnetic actuated mode (MAC-Mode). Silicon cantilevers with a thin magnetic coating on the backside were used (MAC Levers Type II, Agilent) purchased from Lot-Oriel GmbH (Darmstad, Germany) with nominal spring constant of 2.8 N/m. The PicoSPM AFM has a sample plate magnetically suspended below its top-down scanner. The liquid cell is a one-piece teflon well which is clipped onto the freshly cleaved mica on the sample plate. 10 µL (10-50 nM) of a solution of DNA nanorings were placed into the liquid cell on the mica, followed by 10 µL (100 mM) of an aq. $NiCl_2$ solution and 10 µL (100 mM, pH 7.5) Tris·HCl-buffer.

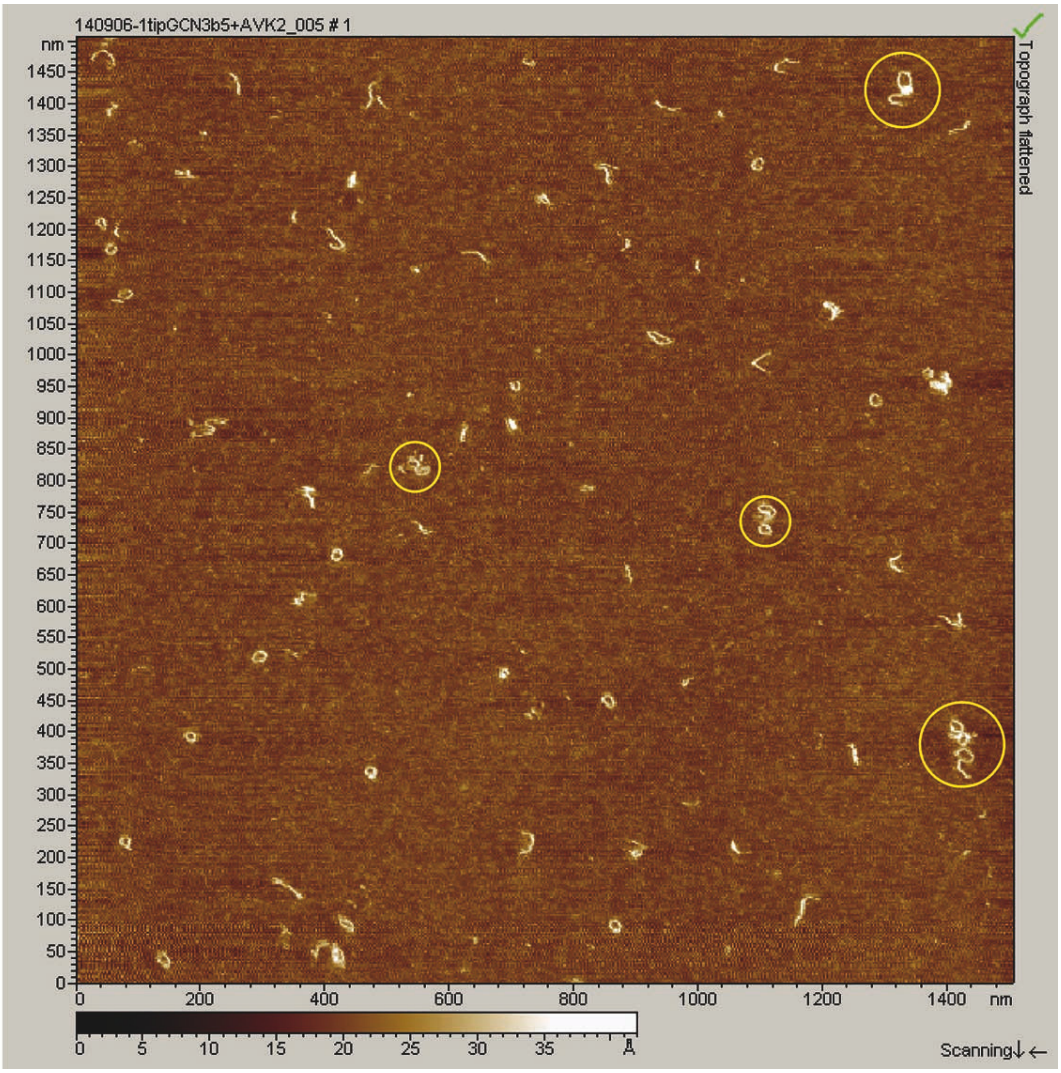
Water was added to reach a volume of 100 µL and the sample was incubated at RT for 20 min. Before scanning another 300 µL (10 mM) Tris·HCl-buffer were added to the liquid cell. The images were processed with WSxM 3.0 beta 9.0 software (Nanotech Electronica, Spain).

AFM imaging methode II (used for dumbbell DNA architectures). AFM images were taken on a Veeco (NY, USA) Dimension 3100 AFM with a Nanoscope IIIa controller in tapping mode. Imaging was performed both, in buffer or in air. 30 µl of a solution of 0.1 mg polyornithin (P3655, Sigma Aldrich) in 1 ml TAE-Mg buffer, the same was used for AFM imaging, was applied on freshly cleaved mica (muscovite grade, Plano, Wetzlar, Germany) and incubated for ca. 2 minutes. The surface was rinsed with milli-Q water and dried with a stream of nitrogen. For imaging, the respective samples were diluted with TAE-Mg buffer to a final concentration of between 2-5 nM. 4 µl of this solution were applied on the pretreated mica surface and were incubated for a minute. For dry imaging the TAE-Mg buffer was briefly washed off with a few ml milli-Q water and immediately dried with a stream of nitrogen. For liquid mode imaging the sample was not washed and dried. 30 µl of TAE-Mg buffer were added both to the sample and on the probe holder. Liquid mode: A Veeco fluid cell (DTFML-DD) was used with Veeco DNP-S tips (0.12-0.58 N/m) in tapping mode. The amplitude set-point was set to 300 mV, typical resonance frequencies of the cantilevers were between 19 and 20 kHz. Scanning frequencies were between 1-2 Hz. Dry mode: ACT probes (25-75 N/m) from APP Nano (Santa Clara, CA, USA) were used for imaging in air. Typical resonance frequencies of the cantilevers were between 300 and 400 kHz. The amplitude set-point was set to 0.3 V, scanning frequencies were between 0.5 to 2 Hz. Prior to imaging the all AFM probes were irradiated with a UV hand lamp for 2-12h from a distance of 1-2 cm. AFM raw data was processed with the Nanoscope(R) 5.31 software (Veeco).



Supplementary Figure S1. Structure of the anthracene modified DNA nano ring. The modified 21-mer DNA sequence is hybridized (but not ligated) to the complementary ss-sequence of the gap ring.

a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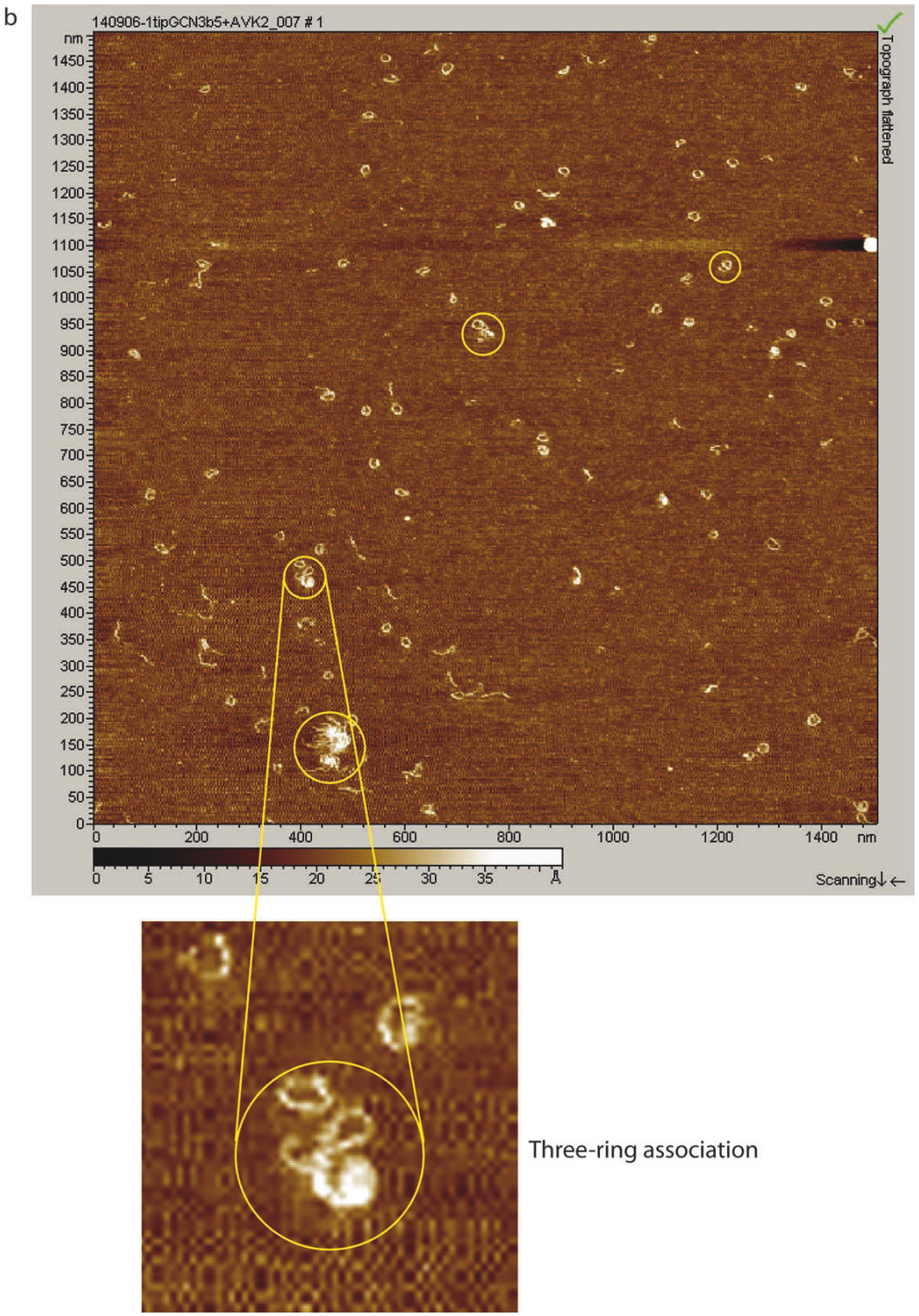
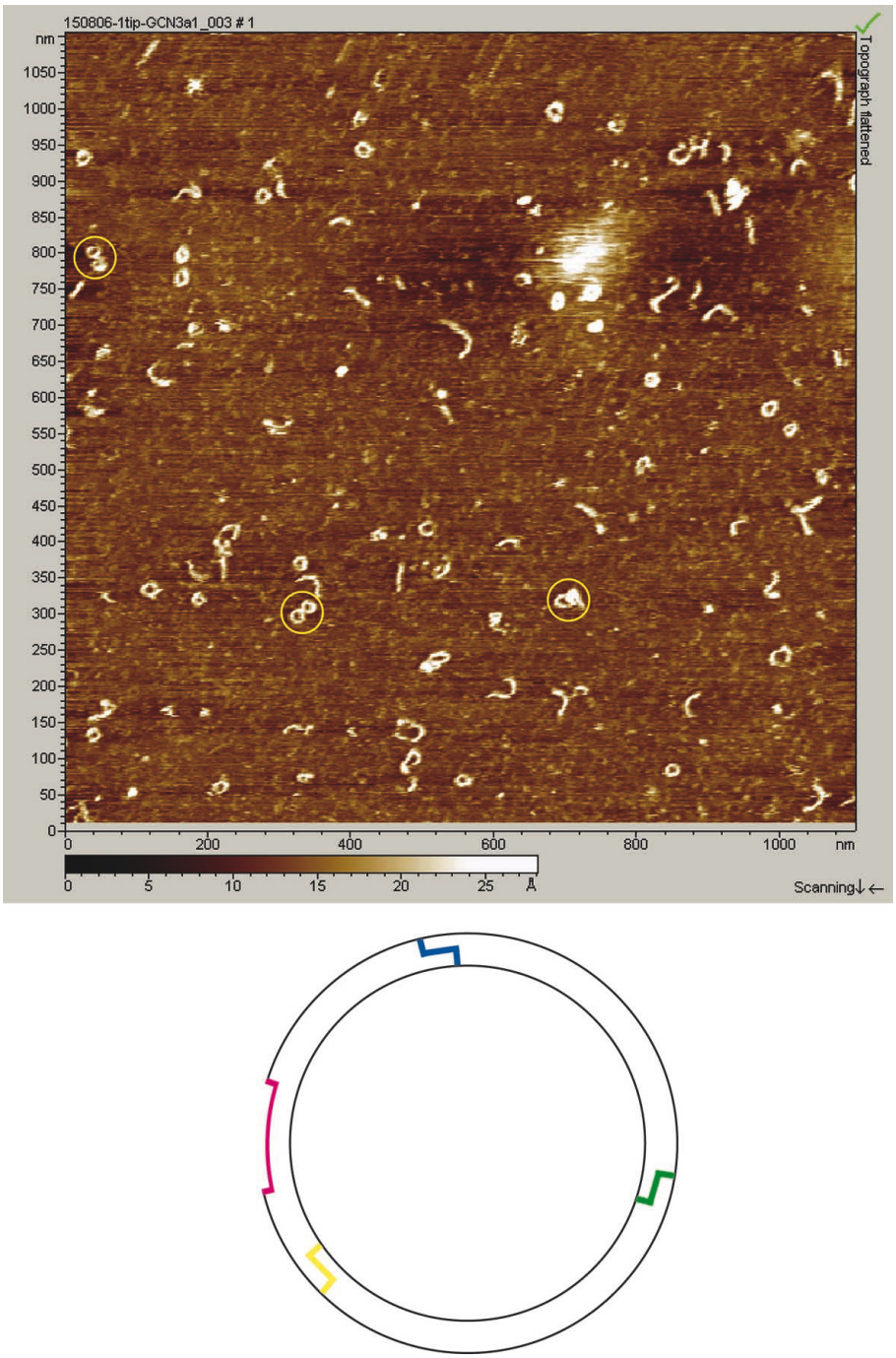
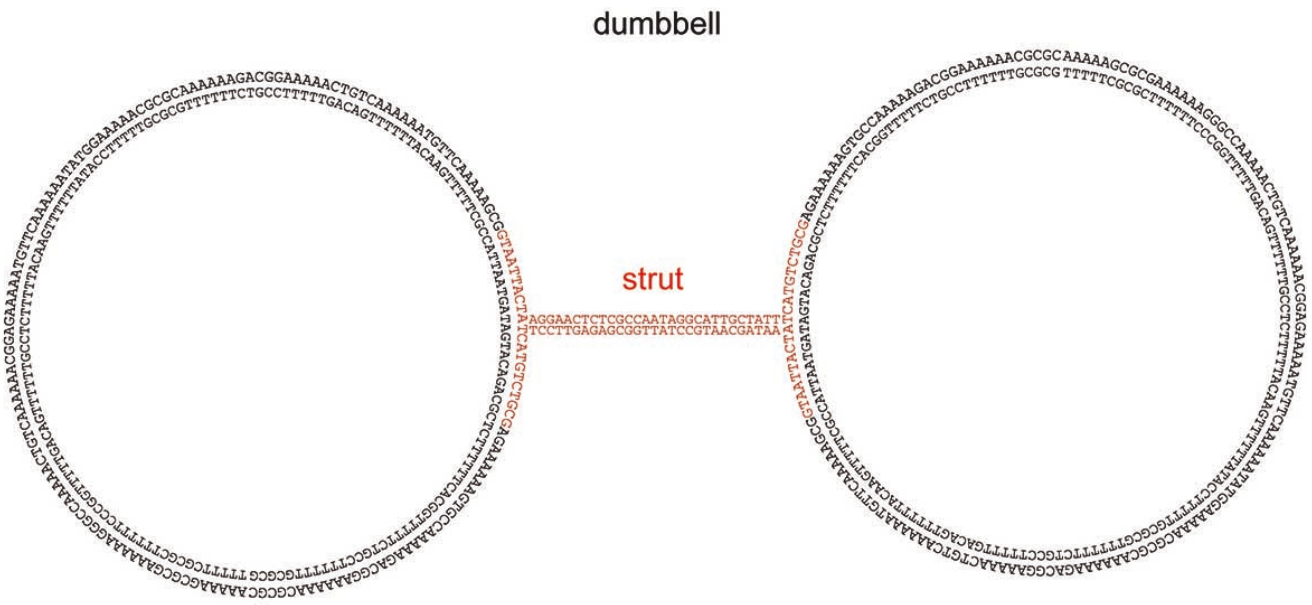


Supplementary Figure S2. AFM scan of additional dsDNA minicircles in the presence of the anthracene-modified ODN. a) AFM scan displaying double- and triple ring aggregates. b) AFM scan displaying double and triple aggregates as well as highly oligomeric clusters.



Supplementary Figure S3. AFM scan of ss-gap containing dsDNA minicircles (bottom panel) in the absence of the anthracene-modified ODN. The AFM scan (top panel) displays few double ring aggregates.



Supplementary Figure S4. Sequences of the gap-ring and strut ODNs used in this study.