Supporting Information

A Novel Family of Structurally Stable Double Stranded DNA Catenanes.

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Buffer systems
1x TAE buffer: 40 mM Tris, 20 mM AcOH, 1mM EDTA.
1x DNA storage buffer: 10 mM Tris·HCl, 50 mM NaCl, 10 mM $MgCl_2$ at pH 7.5.
1x ligase buffer: 40 mM Tris·HCl, 10 mM $MgCl_2$, 10 mM DTT, 5 mM ATP at pH 7.8.
WAX buffer A: 20 mM Tris·HCl, pH 9.0.
WAX buffer B: 20 mM Tris·HCl, 1 M NaCl, pH 9.0.

DNA
Oligodeoxynucleotides (ODNs) were supplied HPLC purified by METABION.

DNA-Nanostructures
The sequences used for synthesizing the catenanes are listed in Table S1, together with the ROs used in this study. The purification was achieved by weak anion exchange HPLC: column TSKgel DEAE-NPR 4.6 mm x 35 mm (TOSOH); WAX buffer B gradient for precursors: from 45% to 65% in 20 min, for catenane structures: isocratic gradient 0 - 10 min at 53% WAX buffer B; then until 28 min from 53% to 62 % WAX buffer B. After purification, the different fractions were concentrated using Amicon Ultra-0.5 centrifugal filter devices (YM-30, Millipore), washed twice with 1x DNA storage buffer and elucidated in 100 µl 1x DNA storage buffer. Concentrations were calculated from the OD values obtained from UV measurements.

Assembly of DNA rings (ring alpha)
The oligonucleotides (4 µM) used for the assembly of the rings (see Figure S1, S2 and Table S1) and NaCl (40 mM) in 1x ligase buffer were annealed from 60 °C to 15 °C over 75 min. Ligase (1 µl/100 µl, 10 U) was added and ligated over night at 15 °C. The products were purified by HPLC and concentrated using Amicon Ultra-30K centrifugal filters.

Assembly of ¾-ring beta
The ¾-ring beta containing all ODNs for the ring beta except the threading-ODN was assembled and purified as described for the DNA rings.

Synthesis of catenanes
1 equivalent of ring alpha was mixed with 1.1 equivalent of threading ODN (in 1x DNA storage buffer supplemented with 10x ligase buffer) and threaded at 15 °C for 3 h. Following

the ¾-ring beta was added and allowed to hybridize for 15 min before ligase was added and ligated over night at 15 °C. In order to convert the non-symmetric pseudocatenane into the catenane, first 10 equivalents of "RO-beta" were added and incubated 30 min at 15 °C before 20 equivalents of "RO-alpha" were added. Again, ligase was added and allowed to ligate over night at 15 °C. The products were purified by HPLC (see supporting Figure S5) and concentrated using Amicon Ultra-30K centrifugal filters.

Gel electrophoresis

The precursors and the products of the catenane assemblies were analysed using agarose gel electrophoresis. 2 % agarose gels (0.5 x TAE) were run for 30 min at 200 V. All gels were poured from 'Agarose High Resolution' (ROTH), run in 0.5 x TAE at 4 °C, stained with ethidium bromide and visualized by UV irradiation.

Atomic Force Microscopy

The measurements were performed on a Nanowizard 3, JPK instruments. The images shown in Figure 2d were performed in "HyperDrive" mode [tapping mode in liquid (ddH_2O)]. The structures were absorbed on freshly cleaved Mica surface coated with poly-ornithine. Ultra Short Cantilever Probes (USC) with high dense carbon tips from NanoWorld were used. All other images were performed in AC mode. The structures were absorbed on freshly cleaved Mica surface coated with poly-ornithine. ACTA probes with silicon tips were used. The images were processed with the JPK processing software.

Pseudocatenane/catenane conversion - followed by fluorescence spectroscopy

The crude Cy3-BHQ2-labeled pseudocatenane (consisting of two 168 bp rings) was excited at 550 nm at 25 °C and the fluorescence emission was measured 3 times at 570 nm for 300 ms. For the pseudocatenane/catenane switch, the sample was treated with TH-RO1 (2 equivalents) to obtain catenane and with Comp-RO1 (2 equivalent related to the TH-RO1) to switch back to pseudocatenane. After addition of the corresponding ROs, the sample was incubated for 30 min at 25 °C before fluorescence was measured.

Pseudocatenane/catenane conversion - followed by gel electrophoresis

Crude pseudocatenane was treated with 5 equivalents of TH-RO1 and incubated for 30 min at 25 °C. An aliquot was taken (lane 1 in Figure 3b), the remaining sample was treated with 10 equivalent of Comp-RO1 and incubated for 30 min at 25 °C and an aliquot was taken (lane 2 in Figure 3b). Before taking another aliquot the sample was treated with 10 equivalents of TH-RO1 and incubated for 30 min at 25 °C (lane 3 in Figure 3b). Finally, 10 equivalent of Comp-RO1 were added, incubated for 30 min at 25 °C and an aliquot was taken. A 2.4 % Agarose gel [poured from 'Agarose High Resolution' (ROTH) in 0.5x TAE] was run for 1.5 h at 160 V, stained with ethidium bromide and visualized under UV irradiation.

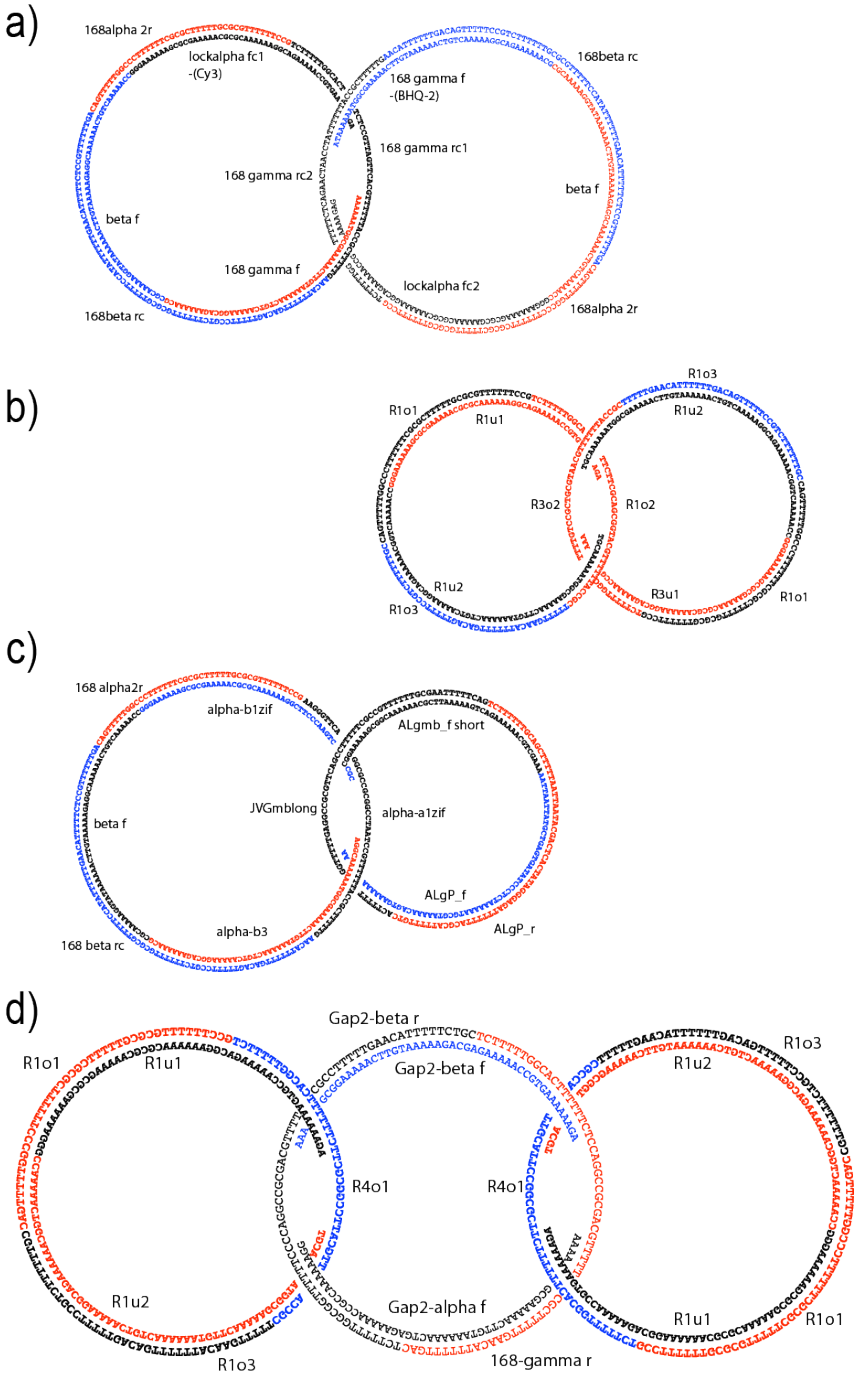

Figure S1. Secondary structure of all catenanes presented in this study, with the corresponding names of the ODNs used for the assembly. a) Pseudocatenane consisting of two 168 bp rings, b) pseudocatenane consisting of two 126 bp rings, c) non-symmetric pseudocatenane consisting of one 168 bp and one 126 bp ring d) pseudo[3]catenane consisting of three 126 bp rings (note that the left and the right ring are identical).

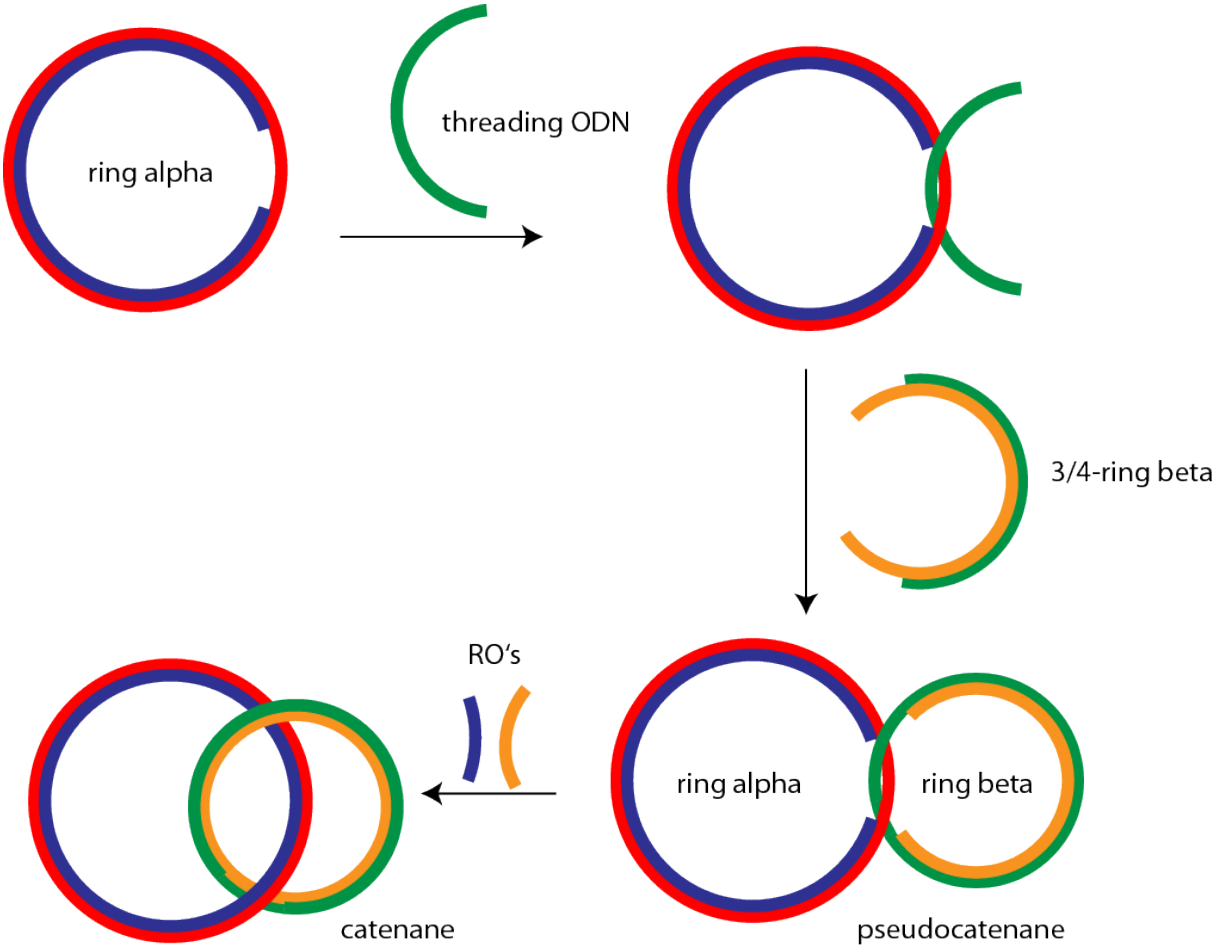

Figure S2. Scheme of the non-symmetric [2]catenane assembly. The rings and ODNs are labelled as described in the synthesis section.

ODN name	Sequence
Catenane ODN's	
R1o1	5'phos-CAGTTTTTGGCCCTTTTTTCGCGCTTTTTGCGCGTTTTTTCCG
R1o2	5'phos-TCTTTTTGGCACTTTTTTCTTCGCAGCGGTACGTTTTTTACCGC
R1o3	5'phos-TTTTTGAACATTTTTTGACAGTTTTTCCGTCTTTTTTGC
R1u1	5'-AGAAAAAAGTGCCAAAAAGACGGAAAAAACGCGCAAAAAGCGCGAAAAAAGGG
R1u2	5'phos-CCAAAAACTGGCAAAAAAGACGGAAAAACTGTCAAAAAATGTTCAAAAAGCGGTAAAAAACGT
168gamma rc1	5'phos-TCTTTTTGGCACTTTTTTCTCCGTTAGTTCACGTTTTTTACCGCTTTTTG
R3u1	5'-AAAAAAGTGCCAAAAAGACGGAAAAAACGCGCAAAAAGCGCGAAAAAAGGG
R3o2	5'-Phos-TCTTTTTGGCACTTTTTTGTCCGCTGCGTAACGTTTTTTACCGC
168Beta rc	5'phos- AACATTTTTTGACAGTTTTTCCGTCTTTTTTGCGCGTTTTTCCATATTTTTTGAACATTTTTCTCCGTTTTTTGA
168Alpha2 r	5'phos-CAGTTTTTGGCCCTTTTTTCGCGCTTTTTGCGCGTTTTTTCCG
Lockalpha fc1-(Cy3)	5'-Cy3-AGAAAAAAGTGCCAAAAAGACGGAAAAAACGCGCAAAAAGCGCGAAAAAAGGG
Beta f	5'phos-CCAAAAACTGTCAAAAAACGGAGAAAAATGTTCAAAAAATATGGAAAAACGC
168gamma rc2	5'phos-TCTTTTTGGCACTTTTTTCTCAGAACTAACCTATTTTTTACCGCTTTTTG
168gamma f-(BHQ-2)	5'phos-GCAAAAAAGACGGAAAAACTGTCAAAAAATGTTCAAAAAGCGGTAAAAAATA-(BHQ-2)
Lockalpha fc2	5'-GAGAAAAAAGTGCCAAAAAGACGGAAAAAACGCGCAAAAAGCGCGAAAAAAGGG
Alfa-b1zif	5'-phos-CGCCCACGCTGAACCCTTCGGAAAAAACGCGCAAAAAGCGCGAAAAAAGGG
Alfa-a1zif	5'phos-AAGGGTTCAGCGTGGGCGCCGCGGCCTAATCCGTTTTTTACCGCTTTTTG
Alpha-b3	5'phos-GCAAAAAAGACGGAAAAACTGTCAAAAAATGTTCAAAAAGCGGTAAAAAACGGAT
JVgmblong	5'-Phos-ACTTTTTTGTGGGTTTTTGAGGCCGCGTTCAGCCTTTTTCGCCGTTTTTTGCGAATTTTTCAG
ALgP_r	5'-Phos- TCTTTTTTGCAGCTTTTTAATTAATACGACTCACTATAGGGAGATTTTTTACGCATTTTTGTC
ALGmb_f short	5'-Phos-AAAGCTGCAAAAAAGACTGAAAAATTCGCAAAAAACGGCGAAAAAGGC
ALgP_f	5'-Phos-AACCCACAAAAAAGTGACAAAAATGCGTAAAAAATCTCCCTATAGTGAGTCGTATTAATTAA
R4o1	5'phos-TCTTTTTGGCACTTTTTTCTTCGCGGCCTTACGTTTTTTACCGC
Gap2-beta r	5'phos-TCTTTTTGGCGGTTTTTTCCCCAGGCCGCGACGTTTTTTCCGCCTTTTTGAACATTTTTCTGC
Gap2-alpha f	5'-GGAAAAAACCGCCAAAAAGAGTCAAAAAATGTTCAAAAAGCGGTAAAAAA
Gap2-beta f (50)	5'-AGAAAAAAGTGCCAAAAAGAGCAGAAAAATGTTCAAAAAGGCGGAAAAAA
168Gamma r	5'phos-TCTTTTTGGCACTTTTTTCTCCAGGCCGCGACGTTTTTTACCGCTTTTTGAACATTTTTTGAC
RO's	
TH-RO1	5'-TCAGCCGCGTGAACTAACGG
Comp-RO1	5'-CCGTTAGTTCACGCGGCTGA
RO1	5'-CGTGAACTAACGG
RO-alpha	5'-phos-TAGGCCGCGG
RO-beta	5'-phos-TGAACGCGGCCTCAAA

Table S1. Sequences of all ODNs used for the nanostructure assemblies and switching experiments.

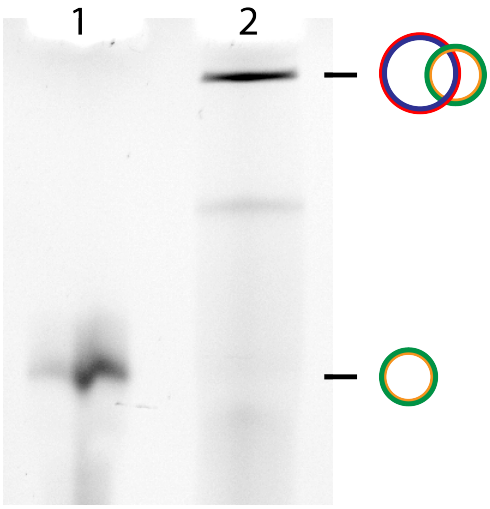

Figure S3. Denaturing PAGE of the 126 bp ring (lane 1) and the non-symmetric catenane (lane 2). As observed in lane 2, the fully double stranded DNA catenated structure stays intact even under denaturing conditions.

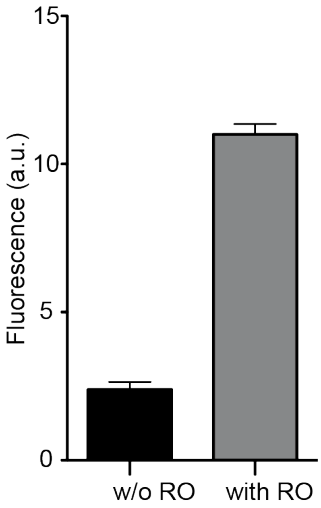

Figure S4. Fluorescence experiment showing the dequenching of the fluorophore due to the pseudocatenane/catenane conversion. Before addition of 10 equivalents of "RO1" to the pseudocatenane consisting of two 168 bp rings the fluorophor and the quencher are in closed proximity resulting in low fluorescence. Addition of the RO leads to dehybridization of the rings and thereby a separation of the fluorophor and the quencher resulting in an increase of fluorescence.

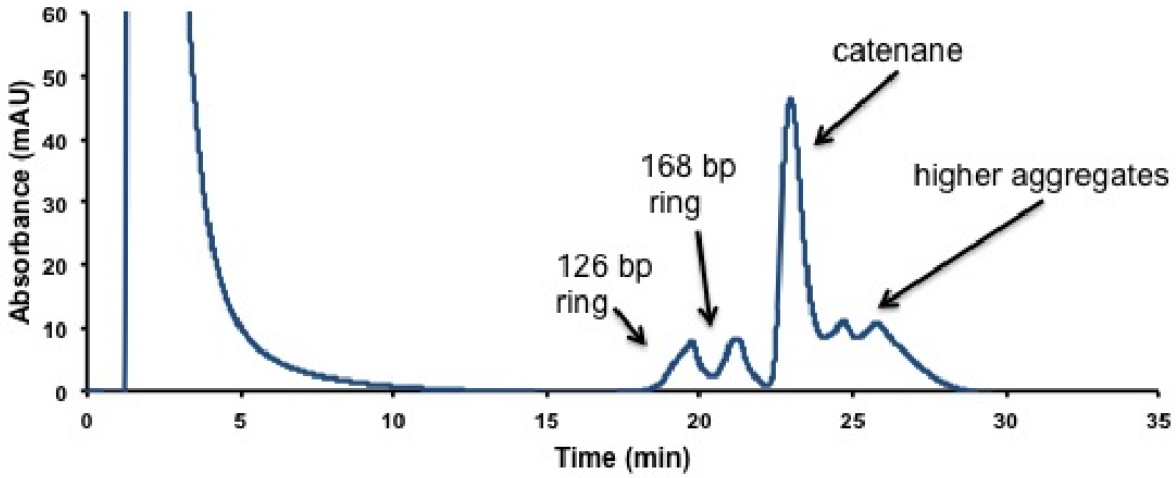

Figure S5. HPLC chromatogram from the purification of the non-symmetric catenane. The UV absorption of the side products/unreacted precursors is indicated in the chromatogram as well as the absorption from the catenane (main product). The high signal at 1-5 min is due to the ATP from the ligase buffer.

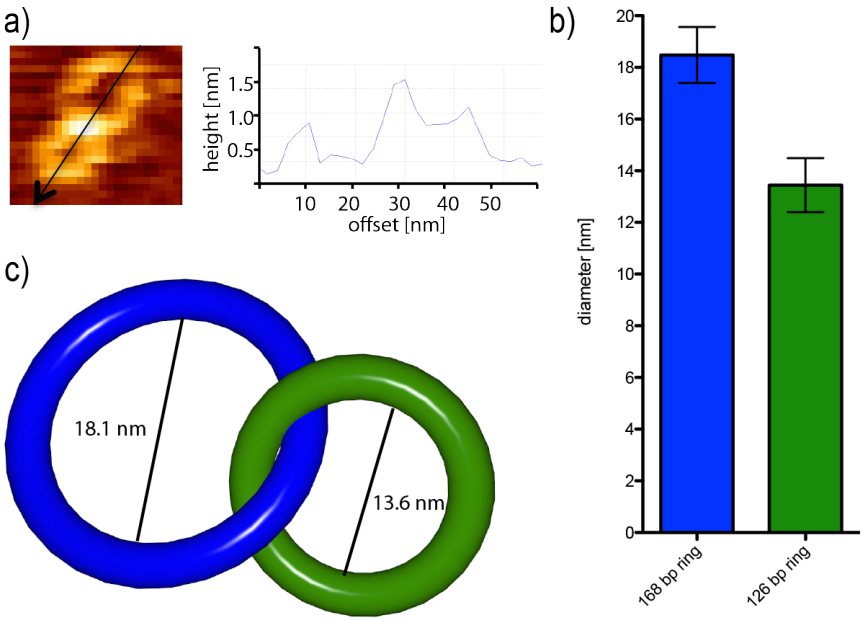

Figure S6. Evaluation of the average size of the catenane rings via AFM. The diameters of 25 different catenane images were measured (as indicated in figure S6a) and an average size of 18.5 nm in the case of the 168 bp ring and 13.4 nm in the case of the 126 bp ring was found [standard deviation of 1 nm (Figure S6b)]. These values agree well with the calculated ones (Figure S6c).

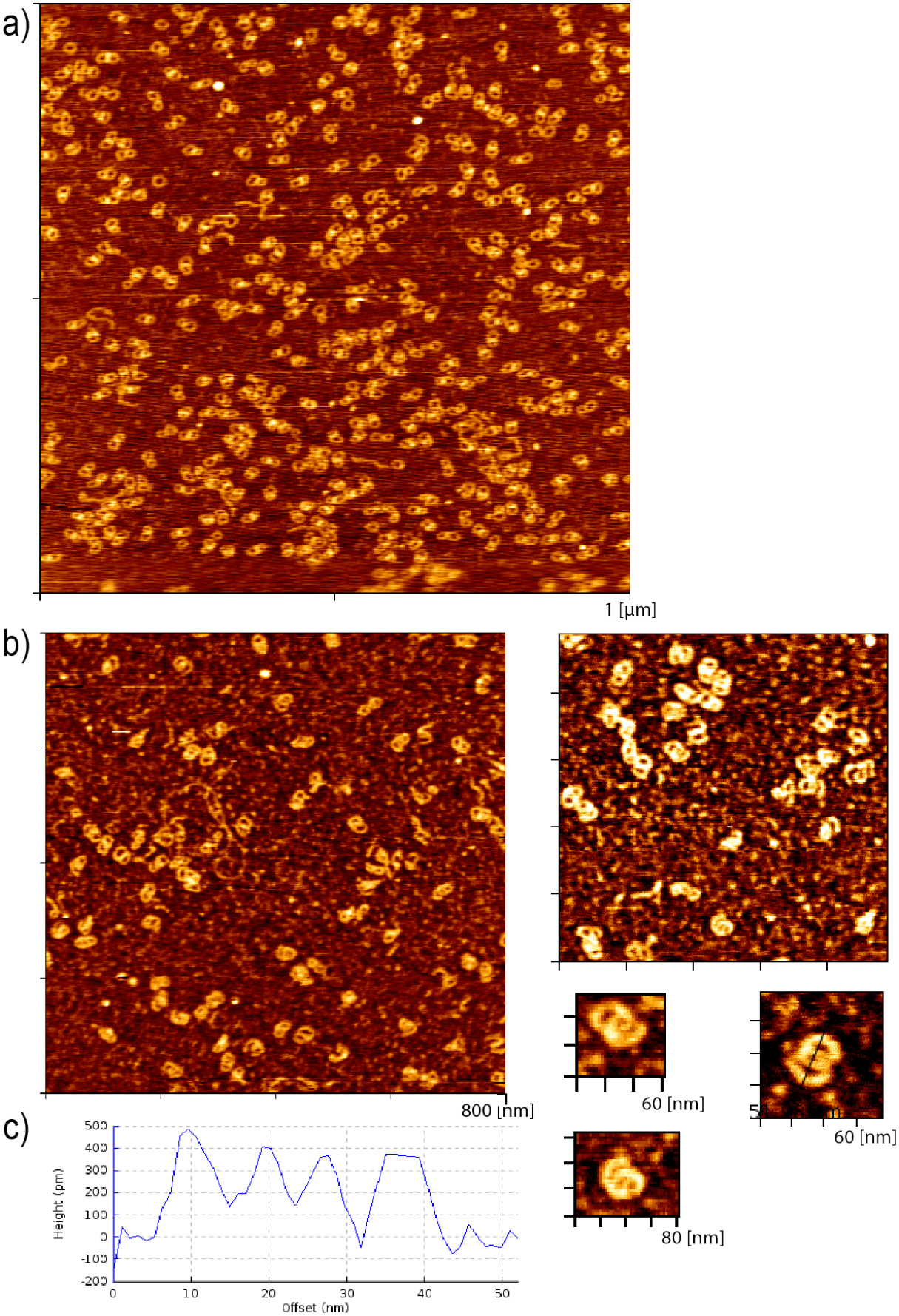

Figure S7. a) 1µm^2 AFM image (intermittent contact mode in air) of the non-symmetric catenane. b) AFM images of the pseudo[3]catenane (intermittent contact mode in air). c) Height profile corresponding to the [3]catenane (Figure S7b, right).