



Supporting Information

One-Step Formation of “Chain-Armor”-Stabilized DNA Nanostructures**

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Supporting Information

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1. General methods

All buffers were stored at room temperature.

10x TE Buffer pH 8 (Tris 100 mM, EDTA 10 mM): 12.11 g Tris (VWR) and 3.72 g EDTA (VWR) were added to 900 ml ddH₂O. The mixture was stirred at room temperature until the solution was clear and homogenous. The pH was adjusted to pH 8 using a 1M HCl solution. Afterwards the solution was filled up to 1 L with ddH₂O.

2M MgCl₂ Solution: 40.66 g MgCl₂ (hexahydrate, Carl Roth) were added slowly to 100 ml ddH₂O (exothermic reaction). The solution was stirred at room temperature until it became clear

10x TE Buffer, 200mM MgCl₂: 9 ml of 10x TE buffer was transferred into a 15 ml falcon tube and 1ml of 2M MgCl₂ was added. The mixture was thoroughly stirred.

1x TE Buffer with 20mM MgCl₂ (*folding buffer*): the buffer was prepared using ddH₂O.

10x TBE Buffer pH8 (Tris-Cl 1M, H₃BO₃ 1M, Na-EDTA 20mM): 121.1 g Tris-Cl (Carl Roth), 61.8 g H₃BO₃ (Carl Roth) and 7.44 g Na-EDTA (Carl Roth) were added to 900 ml ddH₂O. The mixture was stirred at room temperature until the solution was clear and homogenous. The pH was adjusted to pH 8 using a 1M HCl solution. Afterwards the solution was filled up to 1 L with ddH₂O.

0.5x TBE Buffer: the buffer was prepared using ddH₂O.

0.5x TBE Buffer containing 11mM MgCl₂: To 100 ml 10x TBE Buffer pH 8, 11 ml 2M MgCl₂ were added. The mixture was filled up to 2 L with ddH₂O.

2% Agarose gel (0.5x TBE Buffer, 11mM MgCl₂): 2.6 g Agarose (Carl Roth) were dissolved in 130 ml of 0.5x TBE buffer and the mixture was heated up to dissolve completely the agarose. A solution of MgCl₂ (715 µl, 2M) was added to the warm mixture and stirred for a while before casting the gel. To obtain better electrophoretic resolutions, thinner gels were casted if required (1 g agarose dissolved in 50 ml 0.5x TBE buffer + 275 µl 2M MgCl₂). Appropriate sample amounts were loaded into the agarose gel using 2 µl 6x Loading Dye (New England Biolab). The running chamber was filled up with 0.5x TBE buffer containing 11mM MgCl₂. A 70 V voltage was applied to the electrophoretic equipment (BioRad) for 2 hours, while the chamber was cooled down. Agarose gels were stained with ethidium bromide (0.25 µmol/l in 0.5x TBE buffer containing 11mM MgCl₂) for 20 minutes and then destained for 10 minutes in 0.5x TBE buffer containing 11mM MgCl₂. Gels were imaged using a UV-transilluminator (biostep) or Gel Doc Ez System (Biorad).

2% Agarose gel missing MgCl₂ (0.5x TBE Buffer): 2.6 g Agarose (Carl Roth) was dissolved in 130 ml of 0.5x TBE buffer and the mixture was heated up to dissolve completely the agarose. To obtain better electrophoretic resolutions, thinner gels were casted if required (1 g agarose dissolved in 50 ml 0.5x TBE buffer). Appropriate sample amounts were loaded into the agarose gel using 2 µl 6x Loading Dye (New England Biolab). The running chamber was filled up with 0.5x TBE buffer. A 70 V voltage was applied to the electrophoretic equipment (BioRad) for 2 hours, while the chamber was cooled down. Agarose gels were stained with ethidium bromide (0.25 µmol/l in 0.5x TBE buffer) for 20 minutes and then destained for 10 minutes in 0.5x TBE buffer. Gels were imaged using a UV-transilluminator (biostep) or Gel Doc Ez System (Biorad).

10% denaturing polyacrylamide gel electrophoresis (PAGE): A 10% ammonium persulfate (APS, Merck Millipore) solution was prepared in advance dissolving 1 g of APS in 10 ml ddH₂O. Rotiphorese® buffers and gel concentrate (Carl Roth) were used to cast the gel following the supplier instructions.

To initiate the polymerization 150 µl 10% APS solution and 10 µl of N,N,N'',N''-Tetramethylethylenediamine (TEMED, Sigma Aldrich) were added to the gel mixture, stirred and then the gel was casted. Appropriate sample amounts were loaded into the polyacrylamide gel using 2 µl 6x Loading Dye (New England Biolab). The running chamber was filled up with 0.5x TBE buffer. A 120 V voltage was applied to the electrophoretic equipment (Mini-PROTEAN Tetra Cell system, BioRad) for 1 hour. Gels were stained in 200 ml 0.5x TBE buffer containing 20 µl SYBR Gold Stain (Life Technologies) for 10 minutes and imaged using a UV-transilluminator (biostep) or Gel Doc Ez System (Biorad).

TEM imaging: 2 µL sample was adsorbed for 3 minutes onto glow-discharged, carbon-coated TEM grid (PLANO GmbH). The rest of the drop was discarded. The grid was then rapidly washed with 2% aqueous uranyl formate solution containing 25mM NaOH and afterwards stained for 10 seconds with the same solution. Imaging was performed using a JEM 1011 (Jeol) operated at 80 kV (LMU, Physic Department) or a FEI Tecnai G2 Spirit (Bio)twin operated at 80 kV (Aarhus, iNANO).

RP-HPLC analysis: RP-HPLC analysis were obtained by e2695 separation module (Waters) coupled with a 2998 photodiode array detector (Waters). The samples were separated with a reversed phase XBridge OST C18 column (4.6 mm x 50 mm, Waters) with a particle size of 130 Å and an inner diameter of 2.5 µm. The method used is described in table S1.

Table S1. Analytical HPLC method. Buffer A: 100 ml stock solution (101.19 g Triethylamine, 60.05 ml acetic acid HPLC grade dissolved in 1 L H₂O HPLC grade) + 900 ml H₂O HPLC grade. Buffer B: 100 ml stock solution + 100 ml H₂O HPLC grade + 800 ml Acetonitrile HPLC grade. All HPLC grade solvents were purchased from Fischer Chemicals.

t (min)	T (°C)	Flow (ml/min)	Buffer A (%)	Buffer B (%)
0	23°C	1,5	100	0
7	23°C	1,5	55	45
9	23°C	1,5	15	85
10	23°C	1,5	15	85
14	23°C	1,5	100	0
15	23°C	1,5	100	0

UHPLC-LTQ (Orbitrap): mass analysis were obtained using an UltiMate 1290 Rapid Separation LC (RSLC) (Agilent) coupled to an LTQ-Orbitrap XL (Thermo Scientific). A capillary column (Poroshell 120 EC-C18, 4.6, 250 mm, 2.7 µm) with an inner diameter of 0.5 µm was used for the UHPLC-MS analysis and the method applied is described in table S2.

Table S2. UHPLC method. Solvent A: water + 0.1 % (v/v) ammonium carbonate, Solvent B: acetonitrile + 0.1 % (v/v) ammonium carbonate

t (min)	T (°C)	Flow (µl/min)	Solvent A (%)	Solvent B (%)
0	30°C	40	100	0
10	30°C	40	90	10
20	30°C	40	90	10
22	30°C	40	70	30
30	30°C	40	70	30
32	30°C	40	0	100
42	30°C	40	100	0

Fluorescence gel detection: fluorescence detection was carried out using a TECAN LS 300 Laser scanner (laser 496 nm, Filter 590 Cy3, 200 PMT gain, Large pinhole). Bands intensity quantification was made using the software ImageJ.

2. Oligonucleotides

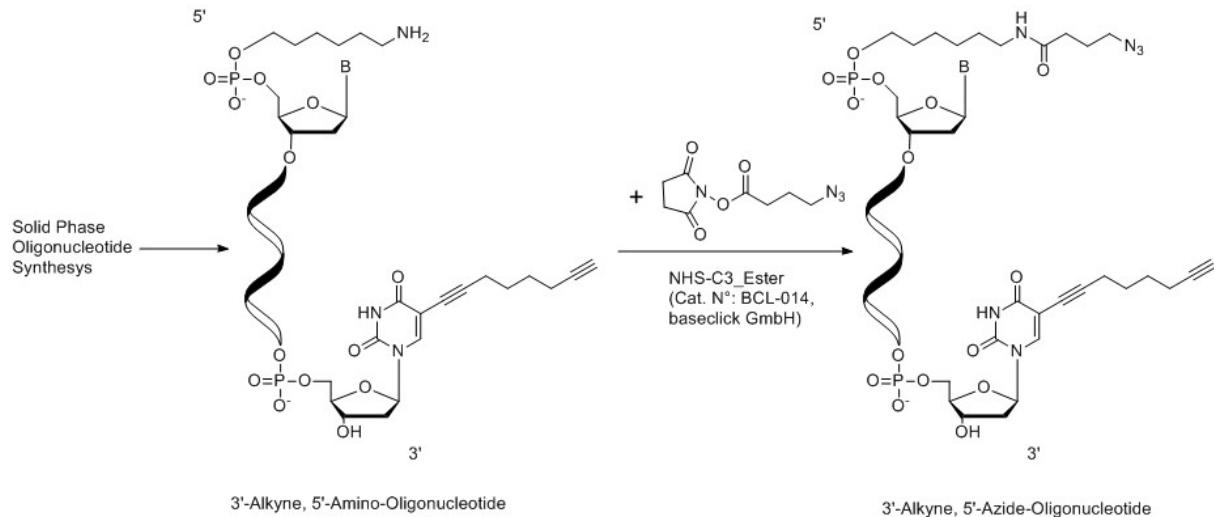
The oligonucleotides in RP-HPLC grade were purchased by baseclick GmbH (Tutzing, Germany) or Metabion GmbH (Munich, Germany).

Unmodified strands

Table S1. Sequences of all unmodified strands used in this work.

Oligo code	Sequence 5' -> 3'	Length (bases)
U1R1	AAA ACGCTAAGCCA CCTTTAGATCC AAAT	28
U1R2	GGTCGTGCGG ACTGTCGAACA CCAACGATGCC TGATAGAAGT	42
U1R3	GCGTGGCAAT AGCCATAAATT CATAACATAACG GCGCCAGACT	42
U1R4	TTTCAAGACC AGCACTTGAT GGCGTAGGGCG GGTTAGCGT	42
U2R1	GGATCTAAAGG ACTTCTATCA AAGACGGGAC GACTCCGGAT	42
U2R2	GGCATCGTTGG AGTCTGGCGC ACGACTTCGA TTTCGGATCCT	42
U2R3	CGTTATGTATG ACGCTAAACC TTGCAATGAC TGAACCTCGAAT	42
U2R4	CGCCCTACGCC AAA AAA GATGGGAGCTT	28
U3R1	AAA ATCCCGGAGTC CGCTGCTGATC AAAT	28
U3R2	GTCCCGTCTT AGGATCCGAAA GCCATAATATA TCGAGACGGT	42
U3R3	TCGAAGTCGT ATTGAGTTCA AATGCTATGC GATGCAGCAT	42
U3R4	GTCATTGCAA AAGCTCCCATC ATTTAATGTCG TTTACAGTAT	42
U4R1	GATCAGCAGCG ACCGTCTCGA CTGCAGAAAT AGGACCCCCAT	42
U4R2	TATATTATGGC ATGCTGCATC TTCCCTGGCAT GGCTGAATTCT	42
U4R3	GCATAGACATT ATACTGTAAC ACCTTACGTA ACTTACAGCCT	42
U4R4	CGACATTAAT AAA AAA GATGAGTATTT	28
U5R1	AAA ATGGGGTCTT CGAGGCAGAAC AAAT	28
U5R2	ATTCTGCAG AGAATTCAAGCC TATTACACATAG GCGAAGGCTT	42
U5R3	ATGCCAGGAA AGGCTGTAAGT TGCATCATGGG GGTCCCTCAAT	42
U5R4	TACGTAAGGT AAATACTCATC CCTGAGTGATC CATGACCCCTT	42
T6-R11	GTTTGCCTCG AAGCCTTCGC CCGCACGACC TGGCTTAGCGT	42
T6-R22	CTATGTGAATA ATTGAGGACC ATTGCCACGC TGTTCGACAGT	42
T6-R33	CCCATGATGCA AAGGGTCATG GGTCTGAAA AATTATGGCT	42
T6-R44	GATCACTCAGG AAA AAA ATACAAGTGCT	28

Modified oligonucleotides (*click-tiles*)



Scheme S1. Synthesis of a generic *click-tile*. The azide group at the 5'-position of the *click-tile* was introduced in a two-step synthesis involving firstly the introduction of a C6-amino linker during the oligonucleotide synthesis followed by the post-synthesis conjugation with the NHS-C3-azide linker. On the other hand, the alkyne group present on the 3'-end of the *click-tile* was directly introduced during the oligonucleotide solid phase synthesis. Both functionalization protocols proceed smoothly, with high yields and are commercially available.

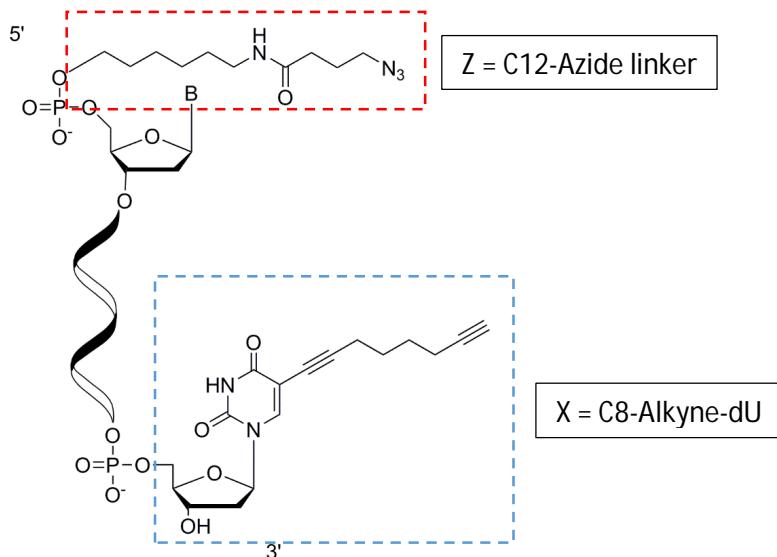


Figure S1. Representation of a generic azide/alkyne modified strand (*click-tile*). In the red box is highlighted the Z modification (C12*-Azide linker conjugated to the 5'-phosphate) and in the blue box the X modification (C8-Alkyne-dU).

* Comparable to a C12 spacer.

Table S2. Sequences of all modified oligonucleotides (*click-tiles*) used in this work. X and Z defined in figure S1.

Oligo code	Sequence	Length (bases)
J1S1	ZAAA ACGCTAAGCCA CCTTTAGATCC AAAX	28
J1S2	ZGGTCGTGCGG ACTGTCGAACA CCAACGATGCC TGATAGAAGX	42
J1S3	ZCGTGGCAAT AGCCATAAATT CATACTAACG GCGCCAGACX	42
J1S4	ZTTTCAAGACC AGCACTTGTAT GGCGTAGGGCG GGTTAGCGX	42
J2S1	ZGGATCTAAAGG ACTTCTATCA AAGACGGGAC GACTCCGGGAX	42
J2S2	ZGGCATCGTGG AGTCTGGCGC ACGACTTCGA TTTCGGATCCX	42
J2S3	ZCGTTATGTATG ACGCTAAACC TTGCAATGAC TGAACCTCGAAX	42
J2S4	ZCGCCCTACGCC AAA AAA GATGGGAGCTX	28
J3S1	ZAAA ATCCCGGAGTC CGCTGCTGATC AAAX	28
J3S2	ZGTCCCGTCTT AGGATCCGAAA GCCATAATATA TCGAGACGGX	42
J3S3	ZTCGAAGTCGT ATTGAGTTCA AATGTCTATGC GATGCAGCAX	42
J3S4	ZGTCATTGCAA AAGCTCCCATC ATTAATGTCG TTTACAGTAX	42
J4S1	ZGATCAGCAGCG ACCGTCTCGA CTGCAGAAAT AGGACCCCCAX	42
J4S2	ZTATATTATGGC ATGCTGCATC TTCCTGGCAT GGCTGAATTX	42
J4S3	ZGCATAGACATT ATACTGTAAA ACCTTACGTA ACTTACAGCCX	42
J4S4	ZCGACATTAAAT AAA AAA GATGAGTATTX	28
J5S1	ZAAA ATGGGGTCCT CGAGGCAGAAC AAAX	28
J5S2	ZATTTCTGCAG AGAATTTCAGCC TATTCACATAG GCGAAGGCTX	42
J5S3	ZATGCCAGGAA AGGCTGTAAGT TGCAATCATGGG GGTCTCAAX	42
J5S4	ZTACGTAAGGT AAATACTCATC CCTGAGTGATC CATGACCTX	42
J6S1	ZGTTTCGCCTCG AAGCCTTCGC CCGCACGACC TGGCTTAGCGX	42
J6S2	ZCTATGTGAATA ATTGAGGAGCC ATTGCCACGC TGTTGACAGX	42
J6S3	ZCCCATGATGCA AAGGGTCATG GGTCTTGAAA AATTATGGCX	42
J6S4	ZGATCACTCAGG AAA AAA ATACAAGTGCX	28

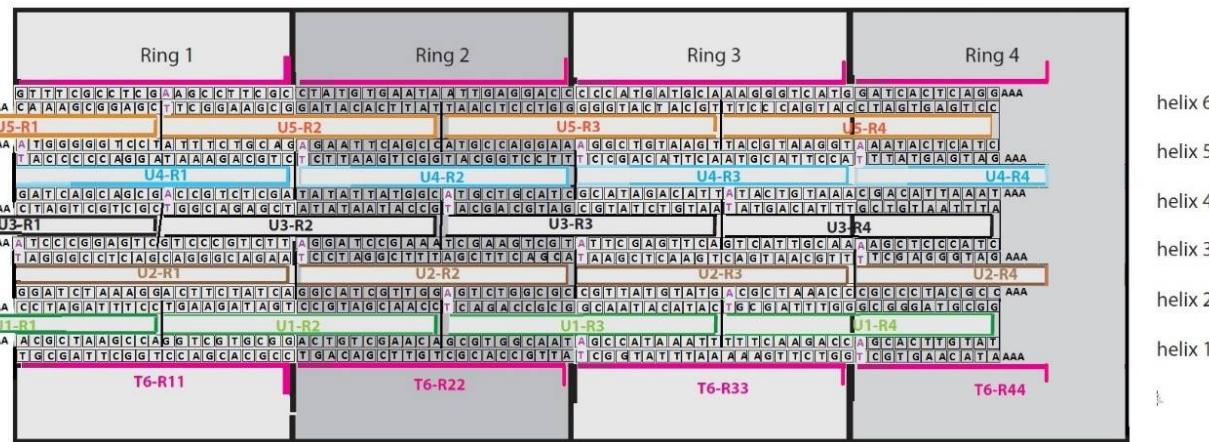


Figure S2. Design of the unmodified tile assembled structure (6HT).^[2] The structure has a calculated length of 27 nm and an outer diameter of 8.5 nm. The helices bow slightly outwards due to the electrostatic repulsion between the negatively charged backbones of the DNA.



Figure S3. Design of the completely modified (M24) tile assembled structure. By inserting modification at 3' and 5' ends there are no changes in the dimension of the structure.

3. Tubes folding

Table S3. Schematic representation of oligonucleotides composition of the different tubes used in the work. To obtain different catenane patterns, selected unmodified oligonucleotides were replaced in the 6HT design with *click*-tiles (highlighted by colored boxes).

6HT				M2				M7			
U5R1	U5R2	U5R3	U5R4	U5R1	U5R2	J5S3	U5R4	U5R1	J5S2	U5R3	U5R4
U4R1	U4R2	U4R3	U4R4	U4R1	U4R2	J4S3	U4R4	U4R1	J4S2	U4R3	U4R4
U3R1	U3R2	U3R3	U3R4	U3R1	U3R2	U3R3	U3R4	U3R1	U3R2	J3S3	U3R4
U2R1	U2R2	U2R3	U2R4	U2R1	U2R2	U2R3	U2R4	U2R1	U2R2	J2S3	U2R4
U1R1	U1R2	U1R3	U1R4	U1R1	U1R2	U1R3	U1R4	U1R1	U1R2	U1R3	J1S4
T6-R11	T6-R22	T6-R33	T6-R44	T6-R11	T6-R22	T6-R33	T6-R44	T6-R11	T6-R22	T6-R33	J6S4
M11				M20				M24			
U5R1	J5S2	U5R3	J5S4	J5S1	J5S2	J5S3	J5S4	J5S1	J5S2	J5S3	J5S4
U4R1	J4S2	J4S3	U4R4	J4S1	J4S2	J4S3	J4S4	J4S1	J4S2	J4S3	J4S4
U3R1	U3R2	J3S3	U3R4	J3S1	J3S2	J3S3	J3S4	J3S1	J3S2	J3S3	J3S4
U2R1	J2S2	J2S3	U2R4	J2S1	J2S2	J2S3	J2S4	J2S1	J2S2	J2S3	J2S4
U1R1	J1S2	U1R3	J1S4	J1S1	J1S2	J1S3	J1S4	J1S1	J1S2	J1S3	J1S4
J6S1	T6-R22	T6-R33	J6S4	T6-R11	T6-R22	T6-R33	T6-R44	J6S1	J6S2	J6S3	J6S4

Folding mixture

Accordingly to table S5 each strand (1 μ l, 0.05 nmol) was added to 10 μ l 10x TE Buffer containing 200mM MgCl₂ and completed with ddH₂O up to 100 μ l. This premix was briefly mixed and folded using a thermocycler (MJ Mini, BioRad). The final concentration of the tube was 0.5 μ M.

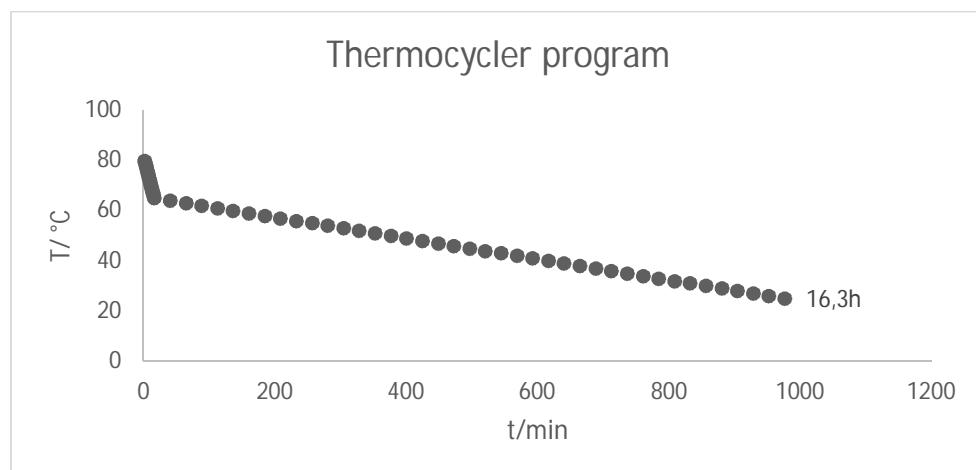


Figure S4. Graphical diagram of the folding program used in this work. The folding program consist of a cooling ramp from 80°C to 65°C, with a decrement of 1° per minute and then from 64°C to 25°C with a decrement of 0.5°C each 12 minutes.

Agarose gel of 6HT and M2 tubes

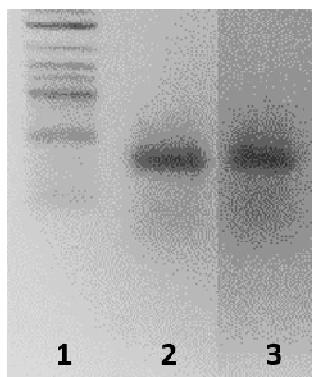


Figure S5. 2% Agarose gel (0.5x TBE Buffer, 11 mM mgCl₂) of 6HT and M2-tube after folding (*lane 1*, 2-Log DNA ladder, *lane 2*, 6HT, *lane 3*, M2-tube). The insertion of two *click*-tiles does not affect the folding process since no shift of the bands was detectable in the agarose gel electrophoresis analysis.

For agarose gel electrophoresis analysis of M7, M11, M20 and M24 see Figure S12.

4. Click reaction

Tubes treated with *click* reaction reagents are additionally marked with an asterisk (e.g. M2-tube becomes M2* tube after *click* reaction).

For a preliminary click optimization, different parameters (Cu(I) sources, temperatures and times) were tested on M2-tube. 6HT was used as negative control. Click reactions were carried out using two different copper (I) sources: CuBr (baseclick) and a heterogeneous catalyst included in a labeling kit (OligoClickM-R reload, baseclick), which is stable even in oxygenated aqueous solutions.

CuBr catalyst: 0.1M solution of the Cu(I)-ligand, tris[(1-hydroxypropyl-1H-1,2,3-triazol-4-yl)methyl]amine (THPTA) was prepared using 10 mg THPTA (baseclick) dissolved in 230 µl *Click Solution* (baseclick). CuBr (5 mg, baseclick) was dissolved in 350 µl of *Click Solution* and promptly added to 2 volumes of THPTA 0.1M. The CuBr/THPTA mixture was always freshly prepared and immediately added to the samples (0.5 µM) in a volume ratio of 0.5:1. The reaction mixture was stirred (maximum 200 rpm) in a thermomixer for different time ranges (from 0.5 hour to 5 hours) and at different temperatures (from 4° to 45°C).

OligoClickM-R reload catalyst: Following the supplier instructions, 40 µl of the solution containing the folded nanostructure (0.5 µM) were added to the heterogeneous catalyst pre-impregnated with 15 µl of the water-soluble *Activator* included into the kit. The reaction mixture was gently mixed at defined reaction temperatures and times. The mixture was then transferred in a fresh vial and the heterogeneous catalyst discharged. The sample was analyzed without any further purification step.

The click reaction does not have negative effect on the folding of the structures, as proved by agarose gel electrophoresis (figure S6) and TEM (figure S7)

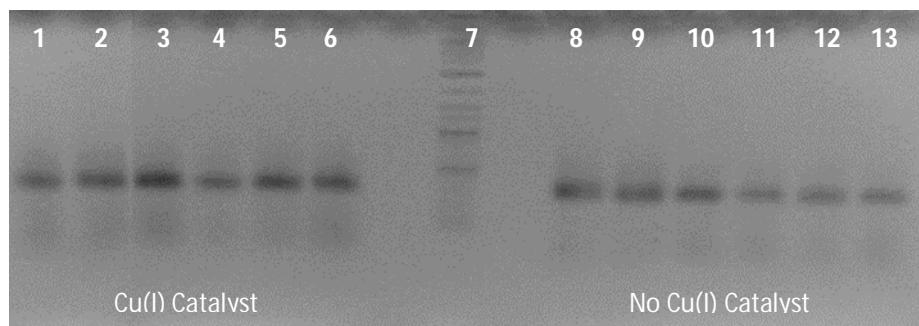


Figure S6. 6HT and M2* tubes after click reaction with baseclick catalyst for 1.5 hours (2% Agarose gel 0.5x TBE buffer, 11mM MgCl₂). From lane 1 to lane 3: 6HT submitted to click reaction at 4°C (lane 1), 20°C (lane 2) and 45°C (lane 3). From lane 4 to lane 6: M2-tube submitted to click reaction at 4°C (lane 4), 20°C (lane 5) and 45°C (lane 6). Lane 7: 2-Log DNA ladder (New England Biolabs). From lane 8 to lane 10: 6HT negative controls (in absence of catalyst) at 4°C (lane 8), 20°C (lane 9) and 45°C (lane 10). From lane 11 to lane 13: M2 negative controls (in absence of catalyst) at 4°C (lane 11), 20°C (lane 12) and 45°C (lane 13).

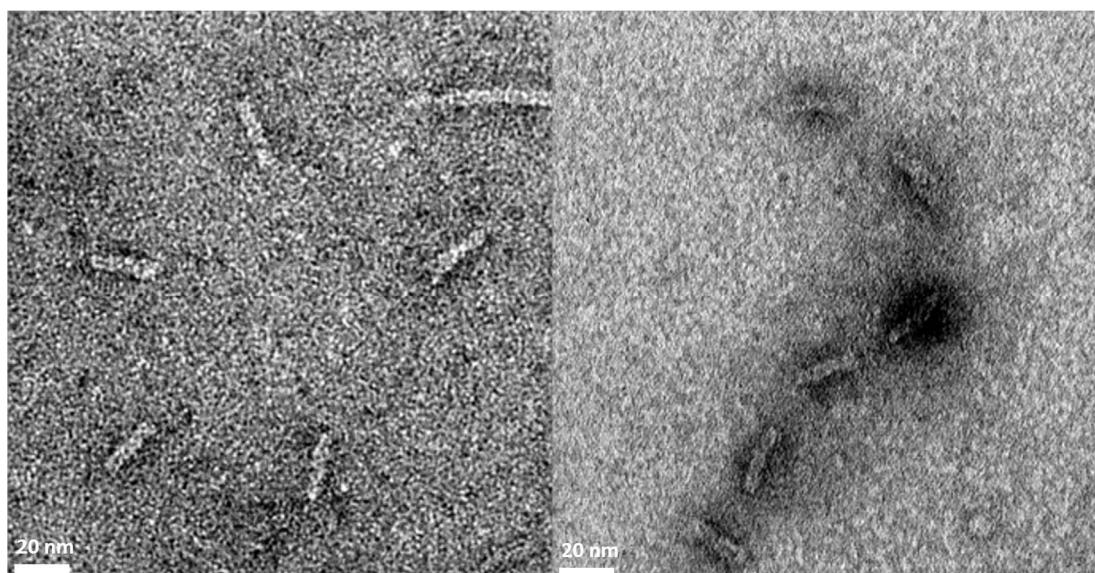


Figure S7. TEM micrographs of M2* tubes. After click reaction (OligoClickM-Reload catalyst, 3 hours, 32°C), the tubes do not look damaged.

TEM micrographs of modified nanotubes after click reaction

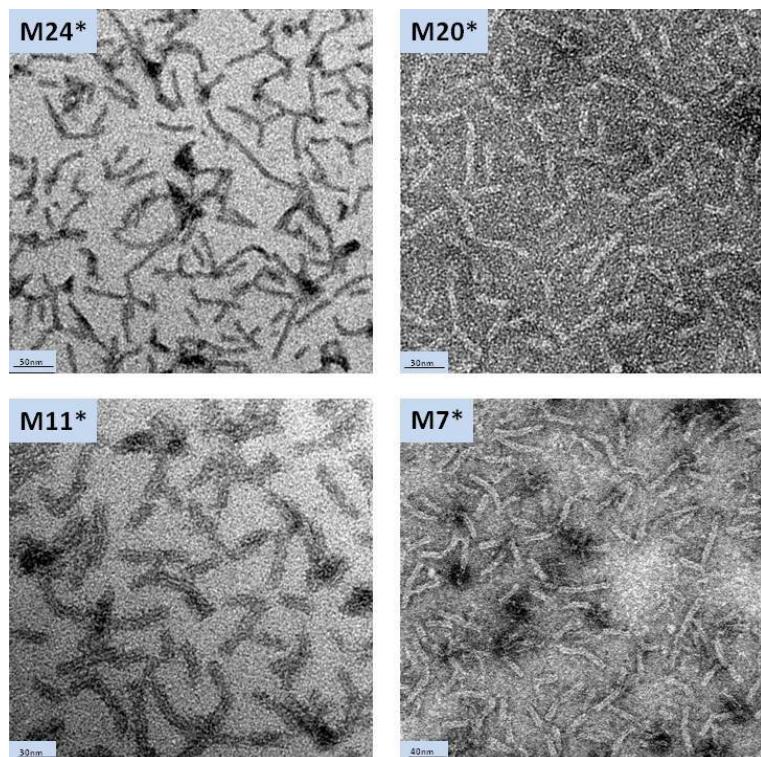


Figure S8. TEM imaging of nanotubes after click reaction. Upper left: M24* tubes, upper right: M20* tubes, bottom left: M11* tubes, bottom right: M7* tubes. All tubes showed no detectable damage in the structure after click reaction (OligoClickM-Reload catalyst, 3 hours, 32°C).

Denaturing PAGE of M2 tubes before and after click reaction

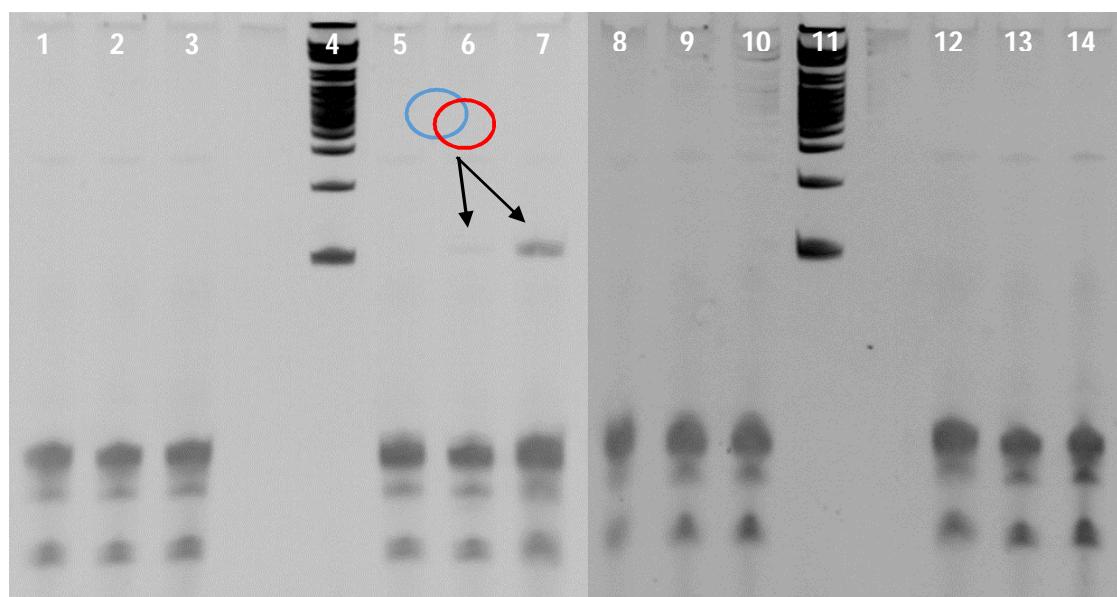


Figure S9. 10% denaturing PAGE analysis of 6HT and M2* tubes after click reaction using the OligoClickM-Reload kit (1.5 hours, different temperatures). From lane 1 to lane 3: 6HT reacted at 4°C (lane 1), 20°C (lane 2) and 45°C (lane 3). Lane 4 and lane 11: 2-Log DNA ladder (New England Biolabs). From lane 5 to lane 7: M2-tubes reacted at 4°C (lane 4), 20°C (lane 5) and 45°C (lane 6). From lane 8 to lane 10: 6HT negative controls (in absence of catalyst) at 4°C (lane 8), 20°C (lane 9) and 45°C (lane 10).

From Lane 12 to lane 14: M2-tubes negative controls (in absence of catalyst) at 4°C (lane 12), 20°C (lane 13) and 45°C (lane 14). Under these conditions (1.5 hours), click reaction occurs only at 20°C (low yield, weak band, *lane 6*) and at 45°C (high yield, stronger band, *lane 7*).

Click reaction optimization

Other tests were performed to find out the best click reaction conditions for this system. CuBr and the heterogeneous catalyst of OligoClickM-Reload kit were compared in a narrower range of temperature (23°C to 32°C) and over different time interval (2.5 hours, 3.5 hours and 5 hours).

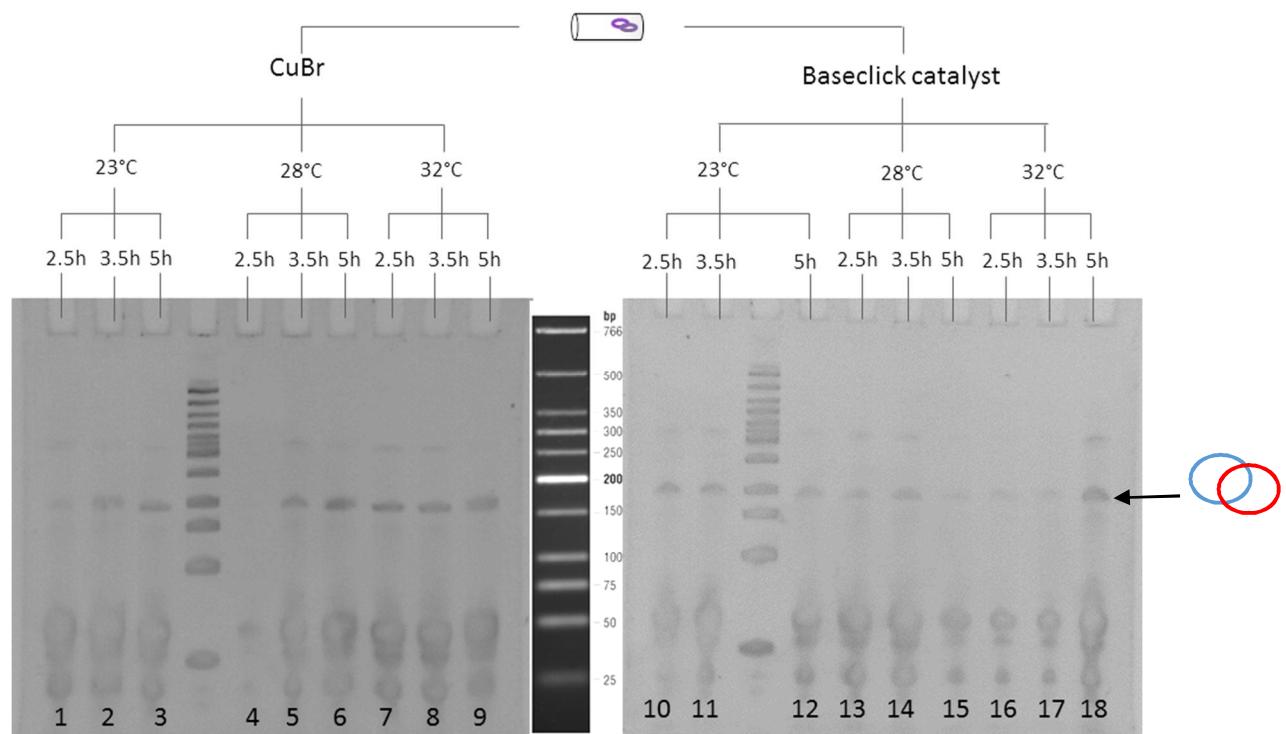


Figure S10. Optimization of click reaction (10% denaturing PAGE). Low molecular ladder (New England Biolabs) was used for this PAGE. The yields of the click reaction (formation of the 2-member ring) were comparable between all the different assays when using CuBr, while the heterogeneous catalyst performed better at 32°C for 5 hours reaction.

Click reaction at 4°C

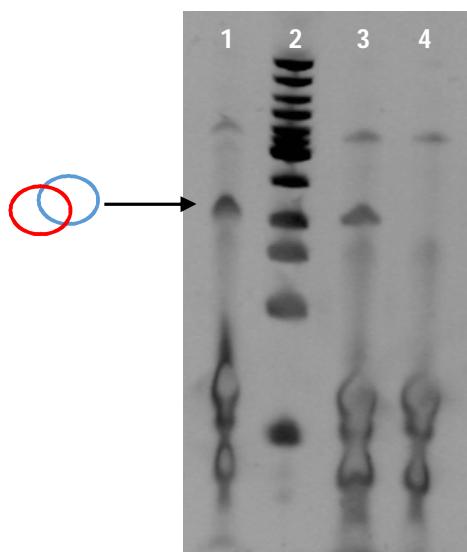


Figure S11. 10% denaturing PAGE of M2-tubes reacted at 4°C using two different Cu(I) sources. Click reaction using CuBr at 4°C (*lane 1*); Low molecular ladder (New England Biolabs, *lane 2*); click reaction using the heterogeneous catalyst at 40°C (*lane 3*) and at 4°C (*lane 4*). At 4°C CuBr catalyst performed better than the heterogeneous catalyst. The latter yielded the 2-member ring at 40°C.

5. Stability of catenane

Reacted and unreacted nanotubes showed similar electrophoretic mobility when loaded into a 2% agarose gel containing divalent cations ($MgCl_2$) as visible in figure S12.

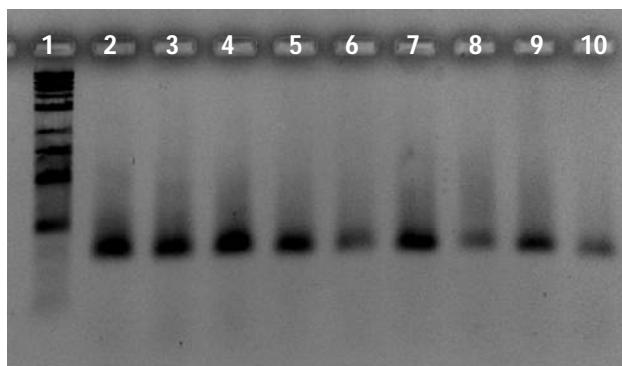


Figure S12. 2% agarose (0.5x TBE Buffer containing 11 mM $MgCl_2$) control gel to test the correct folding of the tubes before and after click reaction. *Lane 1:* 2-Log DNA ladder; *lane 2:* M7* tubes; *lane 3:* M7-tubes; *lane 4:* M11* tubes; *lane 5:* M11-tubes; *lane 6:* M20* tubes; *lane 7:* M20-tubes; *lane 8:* M24* tubes; *lane 9:* M24-tubes; *lane 10:* 6HT.

To prove that DNA catenanes obtained after the click reaction were topologically interlocked, samples were loaded into agarose gel missing $MgCl_2$. Namely, the reacted nanotubes (10 μ l, 900 μ M in *folding buffer*) were loaded into a 2% agarose gel (0.5x TBE buffer) without $MgCl_2$. The corresponding structures before click reaction (M7, M11, M20 and M24) and 6HT were loaded in the same gel as references (Figure S13).

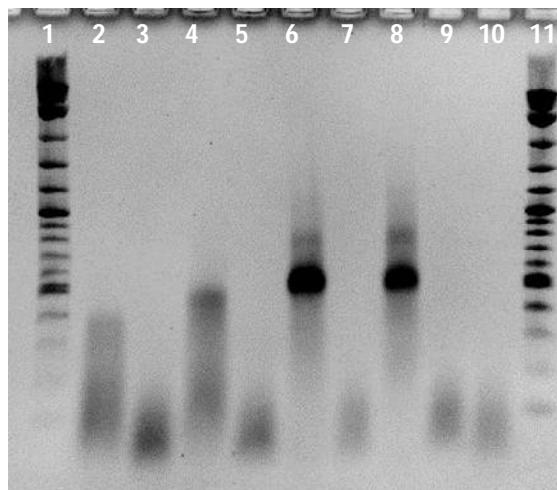


Figure S13. 2% agarose gel (0.5x TBE buffer) missing MgCl_2 to test visualize the DNA catenanes formed after click reaction. *Lane 1:* 2-Log DNA ladder; *lane 2:* M7^* tubes \rightarrow 7-member catenanes; *lane 3:* M7 -tubes; *lane 4:* M11^* tubes \rightarrow 11-member catenanes; *lane 5:* M11 -tubes; *lane 6:* M20^* tubes \rightarrow 20-member catenanes; *lane 7:* M20 -tubes; *lane 8:* M24^* tubes \rightarrow 24-member catenanes; *lane 9:* M24 -tubes; *lane 10:* 6HT; *lane 11:* 2-Log DNA ladder. In agarose gel without MgCl_2 the tubes not submitted to click reaction revealed only a broad band at low molecular weight corresponding to the unfolded strands, while the samples treated with click reagents showed higher molecular weight bands assigned to DNA catenanes.

Resistance to heating (95°C) of DNA catenanes after click reaction

To further test the presence of DNA catenane after the click reaction under denaturing conditions, the reacted and the unreacted samples were heated for 2 minutes at 95°C before loading them into a 2% agarose gel (0.5x TBE buffer) without MgCl_2 .

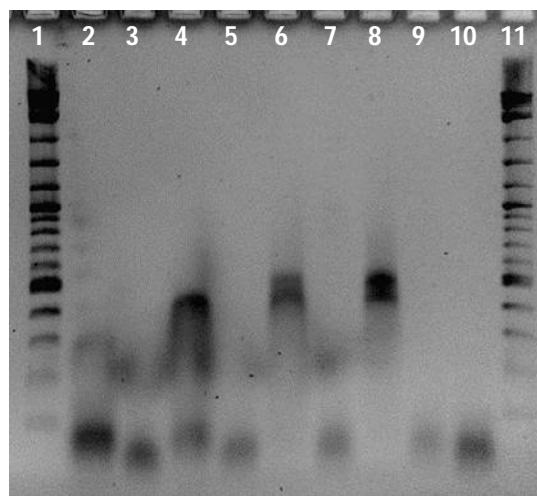


Figure S14. 2% agarose gel (0.5x TBE buffer) missing MgCl_2 of treated and untreated samples after thermal denaturation (2 minutes at 95°C). *Lane 1:* 2-Log DNA ladder; *lane 2:* M7^* tubes \rightarrow 7-member catenanes; *lane 3:* M7 -tubes; *lane 4:* M11^* tubes \rightarrow 11-member catenanes; *lane 5:* M11 -tubes; *lane 6:* M20^* tubes \rightarrow 20-member catenanes; *lane 7:* M20 -tubes; *lane 8:* M24^* tubes \rightarrow 24-member catenanes; *lane 9:* M24 -tubes; *lane 10:* 6HT; *lane 11:* 2-Log DNA ladder. The DNA catenane bands were present for reacted samples confirming the presence of larger entities assigned to DNA catenanes. Unreacted samples unfolded due to the denaturing treatment and the absence of

divalent cation in the gel. The DNA catenane bands are not well defined due probably to the negative effect of MgCl₂ at high temperature.^[3]

Resistance of DNA catenanes to ethanol precipitation

We tested the resistance of the DNA catenanes (present in M7*, M11*, M20* and M24* tubes) to desalting procedure and subsequent ethanol precipitation. The same procedure was carried out on M7, M11, M20, M24 tubes and 6HT as control. Desalting procedure was performed on nitrocellulose membrane (Millipore). Petri dish was filled up with 40 ml ddH₂O, the membrane was placed on the liquid surface and a drop of sample (10 µl) was pipette on it. After 20 minutes, the drop was recollected into a vial containing 10 µL sodium acetate (3M) and mixed thoroughly. Finally 1 ml 100% cold ethanol was added to the mixture and stored at -20°C overnight. The EtOH phases were discharged after centrifugation (20 minutes, 15300 rpm, 4°C) and the pellets were washed once with cold ethanol 70%. At the end of the precipitation procedure, the samples were re-suspended in 30 µl H₂O HPLC grade and analyzed by 2% agarose gel (0.5x TBE buffer) missing MgCl₂. The gel (**Figure 1b** in the manuscript) showed sharp DNA catenanes bands, meaning that the nanostructure submitted to click reaction and thus the DNA catenanes formed within were resistant to this stress procedure, while unreacted samples and 6HT showed only a low molecular band due to the unfolded oligonucleotides.

6. Competitive click reaction

The small fluorescent dye 6-FAM Azide (baseclick) was added to a solution of M2-tubes (10 µl, 5 pmol, 0.5µM) containing two alkynes (10 pmol alkynes). In a first setup a 2.5 times (2.5 µl, 25 pmol) molar excess on respect of total amount of alkynes was used. Alternatively, a 20 times (20 µl, 200 pmol) molar excess was applied. The positive control consisted of a mono-alkyne modified oligonucleotide (5 µl, 5 nmol, 1mM) mixed and reacted with a FAM-azide solution (2.5 µl, 25 nmol, 10mM). The negative control consisted of the M2-tubes (10 µl, 5 pmol, 0.5µM) mixed with the click reaction reagents in absence of any competitive azide. All four samples were submitted to click reaction at 45°C for 1.5 hours using OligoClickM-Reload kit. The reaction mixtures were analyzed afterwards using 10% denaturing PAGE analysis. The efficient formation of 2-member ring catenane is highlighted in figure S15.

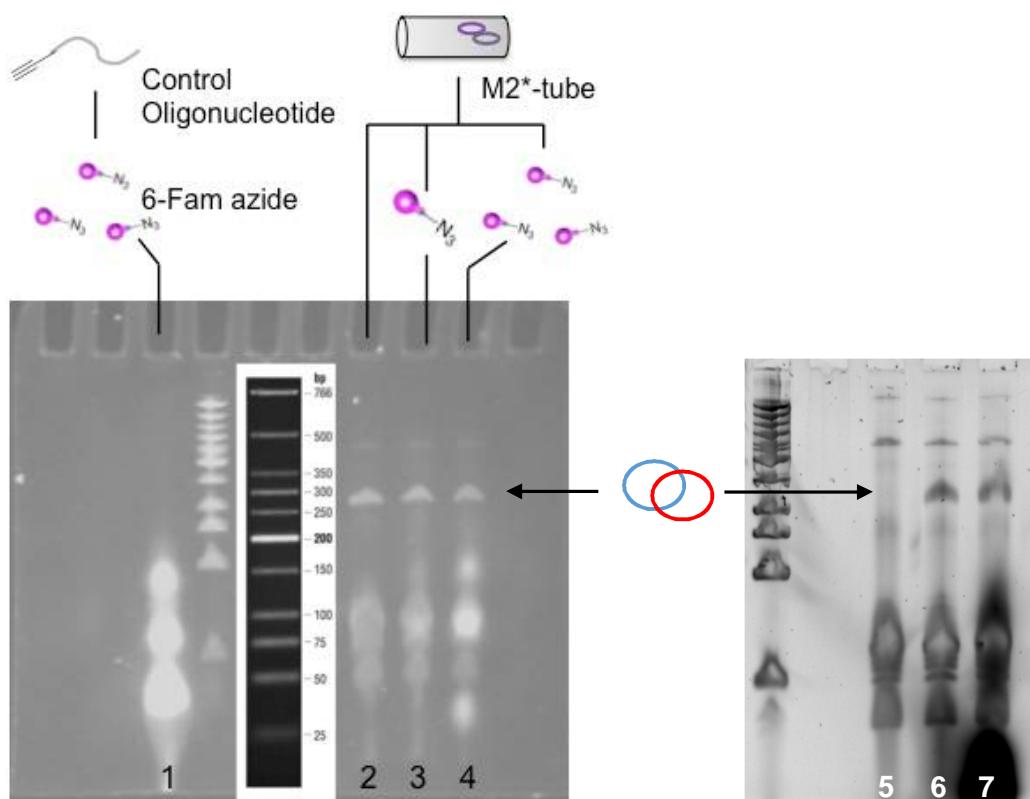


Figure S15. 10% denaturing PAGE of competitive click reaction. *Lane 1:* positive control with mono-alkyne modified oligonucleotides; *Lane 2 and 6:* negative control samples (M2-tubes reacted without 6-FAM azide); *Lane 3:* click reaction on M2-tube in presence of 2.5 equivalents of 6-FAM azide; *Lane 4:* click reaction on M2-tube in presence of 20 equivalents of 6-FAM azide; *Lane 5:* M2-tubes reacted without 6-FAM azide and without copper catalyst; *Lane 7:* click reaction on M2-tube in presence of 1000 equivalents of 6-FAM azide. The ladder used in this gel is Low molecular ladder (New England Biolabs). Although the presence of a large excess of competitor (up to 1000 fold excess) the click reaction between the two *click-tiles* inside the M2-tube was preferred, as proved by the persistence of the band assigned to the 2-member ring catenane.

7. RP-HPLC and MS

Starting from the observation that unmodified oligonucleotides have a different retention time in RP-HPLC from the respective *click*-tiles, we used RP-HPLC as analytical method to get more insight on ring formation efficacy. *Click*-tiles (42mer) introduced in the M2 design and corresponding unmodified oligonucleotides were independently analyzed by RP-HPLC, generating the appropriate references (Figure S16 – S19).

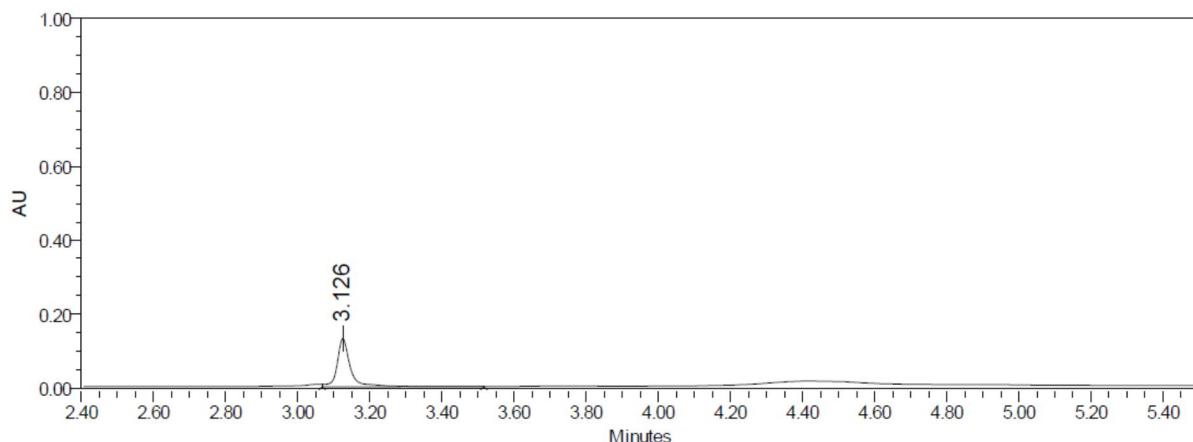


Figure S16. HPLC chromatogram of U4R3 unmodified strand. U4R3 (4 μ L, 50 μ M) was diluted with 16 μ L H₂O HPLC grade (Final concentration 10 μ M – Injection Volume 18 μ L). The unmodified strand U4R3 showed a retention time of 3.12 min.

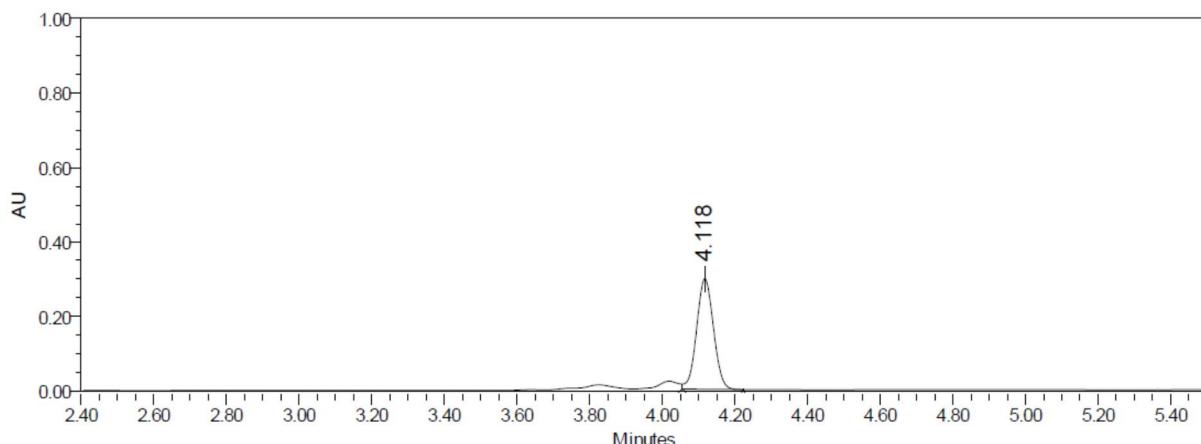


Figure S17. HPLC chromatogram of *click*-tile J4S3. J4S3 (4 μ L, 50 μ M) was diluted with 16 μ L H₂O HPLC grade (Final concentration 10 μ M – Injection Volume 18 μ L). The *click*-tile J4S3 showed a retention time of 4.11 min, 1 minute later than the corresponding unmodified strand U4R3.

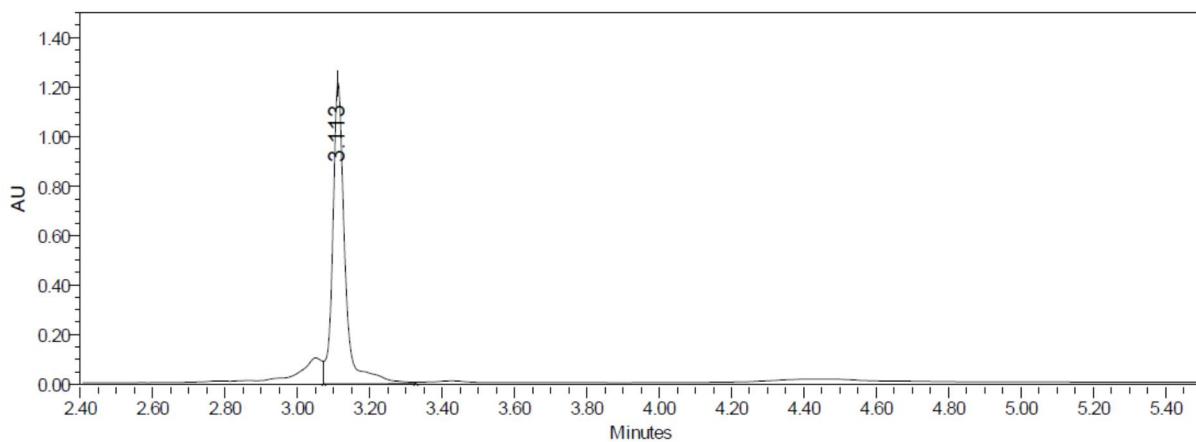


Figure S18. HPLC chromatogram of U5R3 unmodified strand. U5R3 (4 μ L, 50 μ M) was diluted with 16 μ L H₂O HPLC grade (Final concentration 10 μ M – Injection Volume 18 μ L). The unmodified strand U5R3 showed a retention time of 3.11 min.

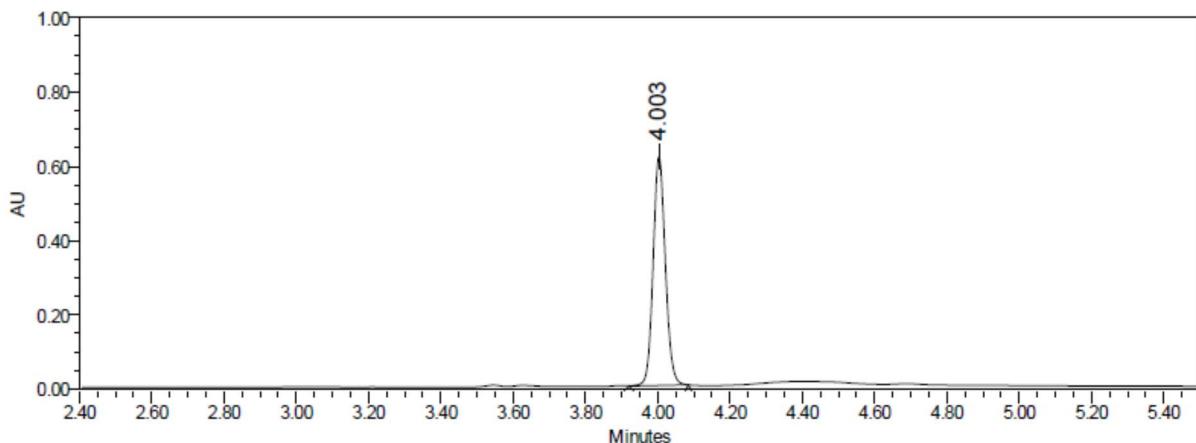


Figure S19. HPLC chromatogram of *click-tile* J5S3. J5S3 (4 μ L, 50 μ M) was diluted with 16 μ L H₂O HPLC grade (Final concentration 10 μ M – Injection Volume 18 μ L). The *click-tile* J5S3 showed a retention time of 4.00 min, 1 minute later than the corresponding unmodified strand U5R3.

The folded structures 6HT, M2-tubes and M2* tubes (each consisting of 24 oligonucleotides) were separately analyzed by RP-HPLC as well (Figure S20 – S22).

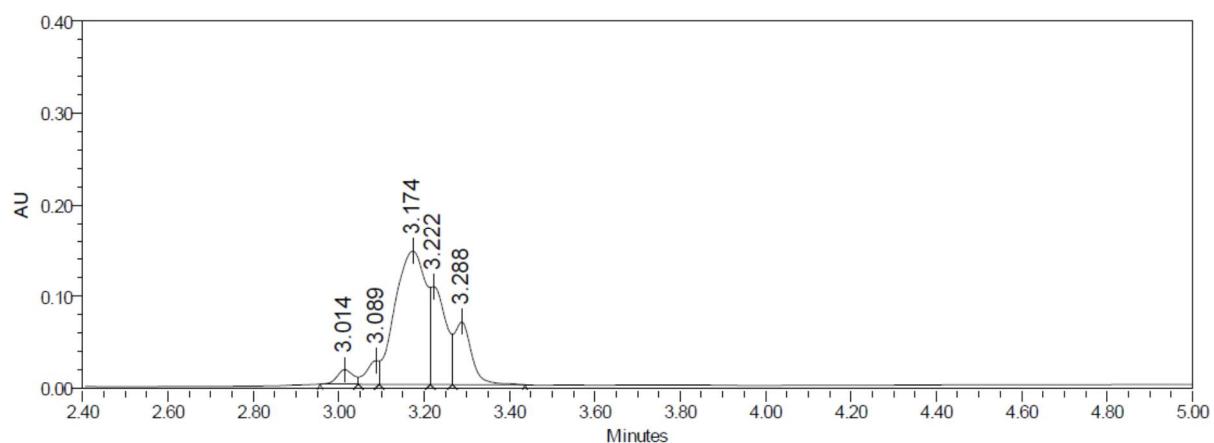


Figure S20. HPLC chromatogram of 6HT. 6HT (20 μ L in *folding buffer*) was diluted to 500 μ L with *folding buffer* and filtrated using Amicon Centrifugal filters (Millipore) following the supplier instructions. Just

before the injection, the recovered sample ($30 \mu\text{L}$) containing the folded tube was submitted to mechanical stress (1 minute vortex and 1 minute ultrasound) to promote denaturation of the nanostructure and eventually a better HPLC resolution ($28 \mu\text{l}$ injection volume). The analysis of the complete strand mixture (6HT) by RP-HPLC lead to a large chromatogram peak in the range between 2.9 and 3.3 minutes.

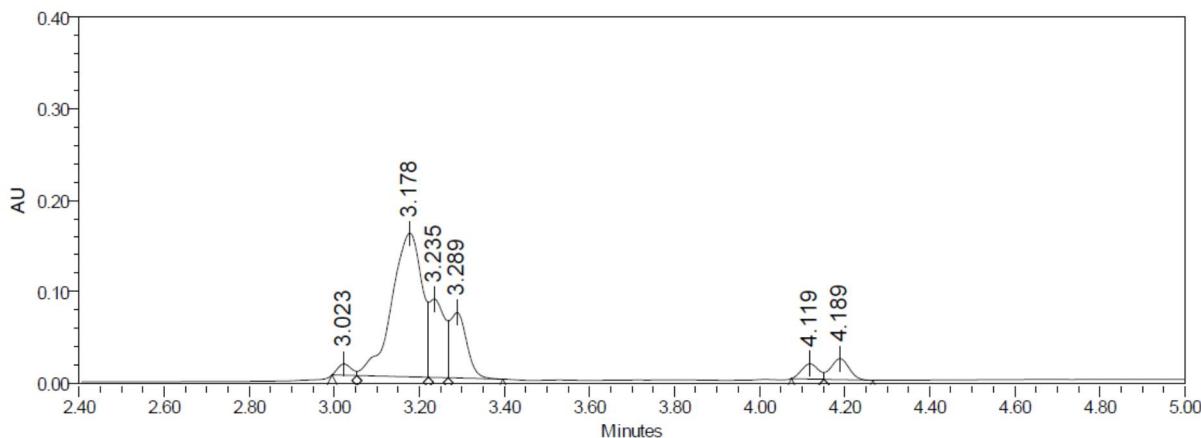


Figure S21. HPLC chromatogram of M2-tubes. M2-tubes ($20 \mu\text{l}$ in *folding buffer*) was diluted to $500 \mu\text{l}$ with *folding buffer* and filtrated using Amicon Centrifugal filters (Millipore). Just before the injection, the recovered sample ($30 \mu\text{L}$) containing the folded tube was submitted to mechanical stress (1 minute vortex and 1 minute ultrasound) to promote denaturation of the nanostructure and eventually a better HPLC resolution ($28 \mu\text{l}$ injection volume). The *click-tiles* were well separated from the rest of unmodified oligonucleotides, thus being easily detectable as double peak at 4.1 minutes.

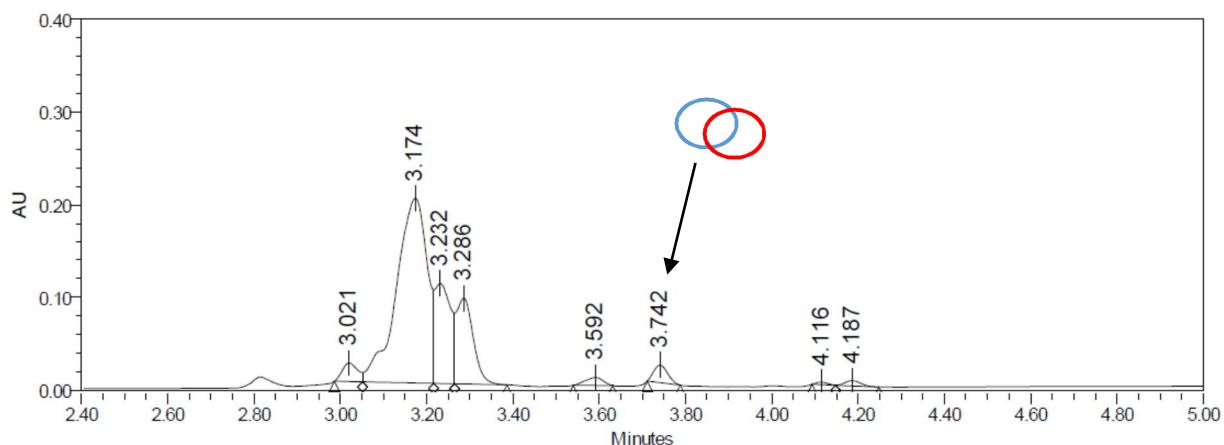


Figure S22. HPLC chromatogram of M2* tubes. M2* tubes ($40 \mu\text{l}$ in *folding buffer*) was diluted to $500 \mu\text{l}$ with *folding buffer* and filtrated using Amicon Centrifugal filters (Millipore). Just before the injection, the recovered sample ($30 \mu\text{L}$) containing the folded tube was submitted to mechanical stress (1 minute vortex and 1 minute ultrasound) to promote denaturation of the nanostructure and eventually a better HPLC resolution ($28 \mu\text{l}$ injection volume). After carrying the *click* reaction on the M2-tube, the HPLC chromatogram showed the formation of a new peak at 3.7 min, which was assigned to the newly formed 2-members ring catenane. The two *click-tiles* (double peak at 4.1 minutes) were clearly consumed during the reaction.

The fraction at 3.7 min containing the isolated two-member ring catenane, collected from analytical HPLC, was concentrated by evaporation in a SpeedVac (Christ AVC 2-25 CD coupled with cooling trap Christ CT 04-50 SR). The dried sample was re-suspended in 50 µl H₂O HPLC grade and analyzed by LC-MS (UHPLC – LTQ, Orbitrap, Thermo Scientific) as showed in figures S23-S26.

Table S4. Calculated value of dimer-ions.

-	M (g/mol)	[M-8] ⁸⁻	[M-9] ⁹⁻	[M-10] ¹⁰⁻
Two-member catenane	26665,36	3332,16	2961,80	2665,52

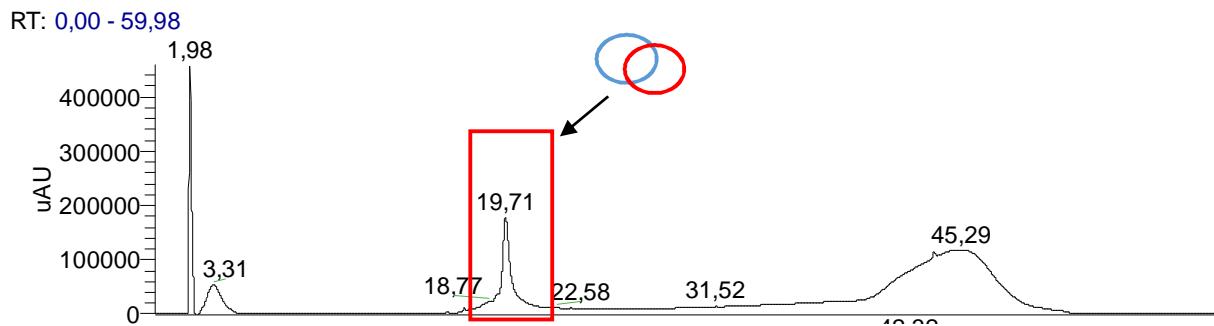


Figure S23. UHPLC chromatogram analysis of isolated 3.7 min HPLC peak. The UHPLC chromatogram showed the peak of the isolated two-member ring catenane (red box) at 19.71 minutes. This peak was further analyzed in the coupled mass spectrometer.

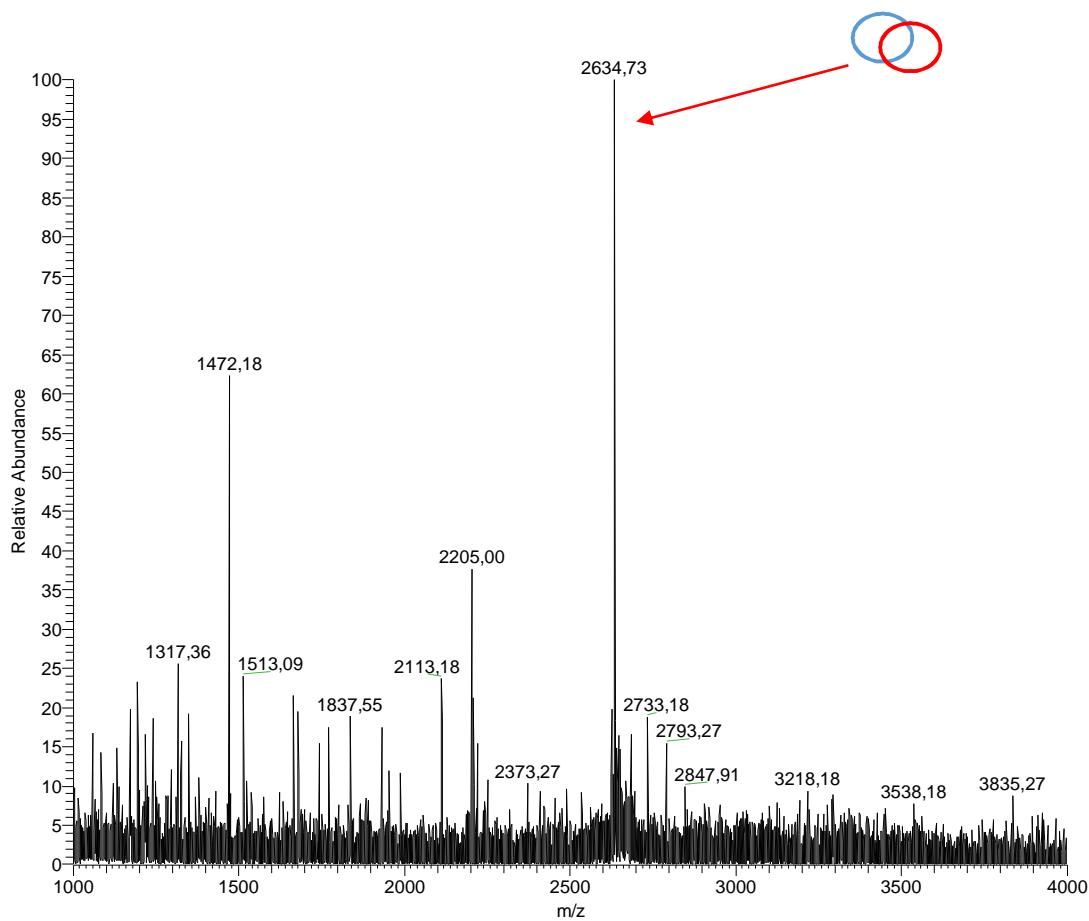


Figure S24. ITMS - pESI full ms spectrum of UHPLC 19.71 min peak. The red arrow indicates the mass of $m/z = [M-10]^{10^-}$. Calcd. 2665.52 m/z. Found: 2634.73 m/z.

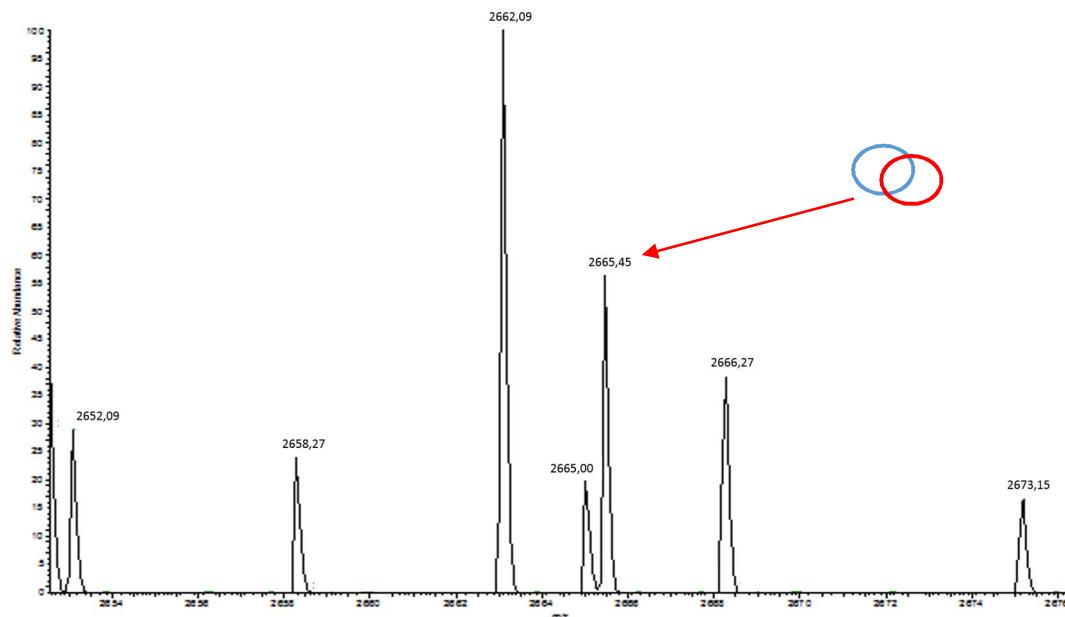


Figure S25. Zoom of spectrum in figure S24 in the region of the mass peak 2634.73. The exact mass of $m/z = [M-10]^{10^-}$ is indicated from the red arrow ($[M-10]^{10^-}$ Calcd. 2665.52 m/z. Found: 2665.45 m/z).

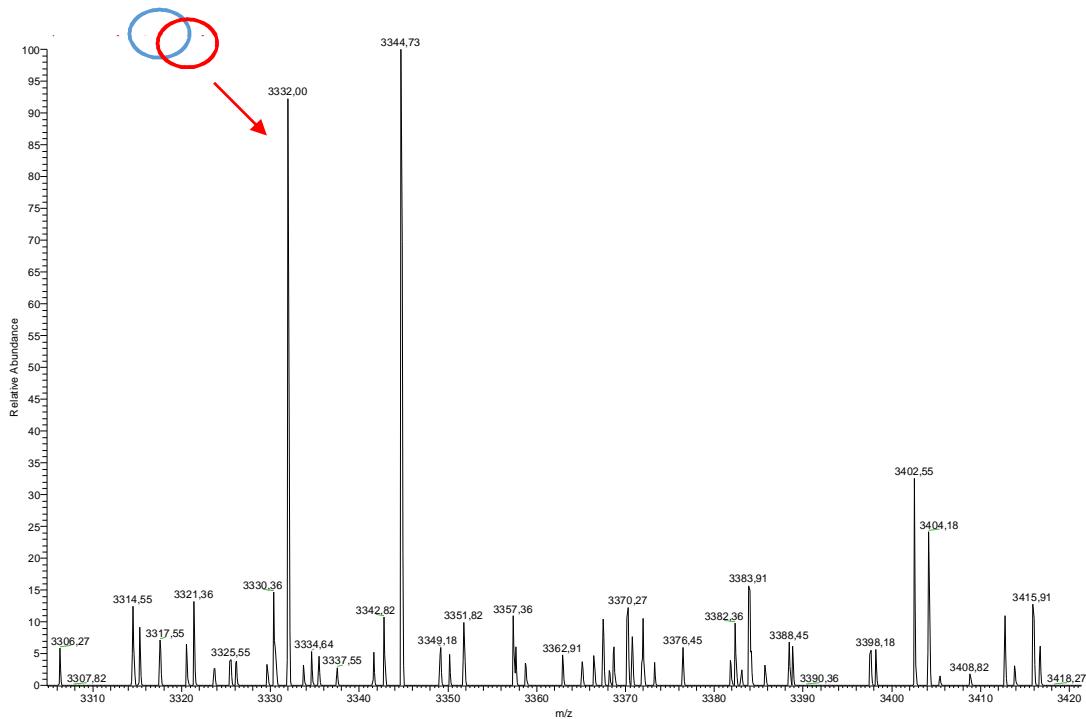


Figure S26. ITMS - pESI full ms spectrum of UHPLC 19.71 min peak. The red arrow indicates the mass of $m/z = [M-8]^{8-}$. Calcd. 3332.00 m/z. Found: 3332.00 m/z).

The results showed the expected masses assigned to the new formed two-member ring catenane. Depurination during electron spray ionization (ESI) mass analysis^[4] could explain some discrepancies in the overall peak analysis.

8. Desalting experiments

Petri dishes were filled up with 40 ml H₂O HPLC grade. Nitrocellulose membranes (Millipore) were individually placed on the water surface of different petri dishes and sample drops (typically 10 μ l) were carefully pipetted on them. After 20 minutes desalted samples (estimated $[Mg^{++}] = 0$) were analyzed by 2% agarose gel electrophoresis and TEM imaging (e.g. figure S28). Alternatively each membrane was transferred to dishes containing buffers with different concentrations of Mg⁺⁺ and dialyzed for further 20 minutes. The samples were re-collected and analyzed by 2% agarose gel electrophoresis (see Figure 2a in the manuscript).

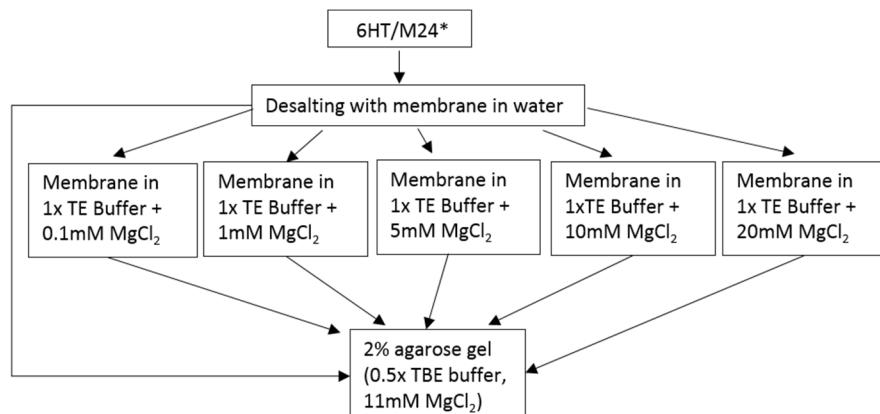


Figure S27. Scheme of the desalting experiments. Nanotubes were subsequently measured in pure water or in buffers containing 0.1, 1, 5, 10 and 20mM MgCl₂ respectively.

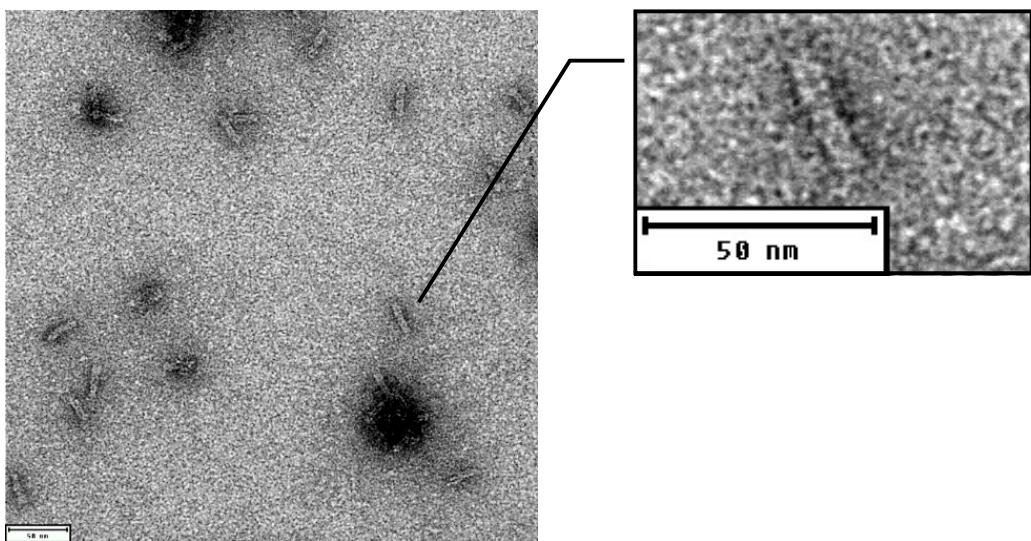


Figure S28. TEM micrograph – and enlargement - of M24* tubes after desalting procedure. M24* tubes are still correctly folded when dissolved in pure water (Calculated nanotube length = 27 nm).

9. Melting profiles^[5]

To better investigate the melting behavior of the tile assembly structures, melting profiles were obtained using a real time PCR machine (LightCycler 480 System, Roche). The tested objects were diluted to a concentration of ca. 20nM in *folding buffer* (20 μ l final volume). As control, a duplex DNA (23mer, 2.4 μ M) was submitted to the same heating ramp. A solution of DNA intercalating dye (SYBRGold 1x, Invitrogen) was added to each sample. The fluorescence was recorded over a heating ramp (25°C – 90°C, 0.06 °C/sec, 10 acquisitions per °C). Data were analyzed using the Light Cycler 480 Software (Roche).

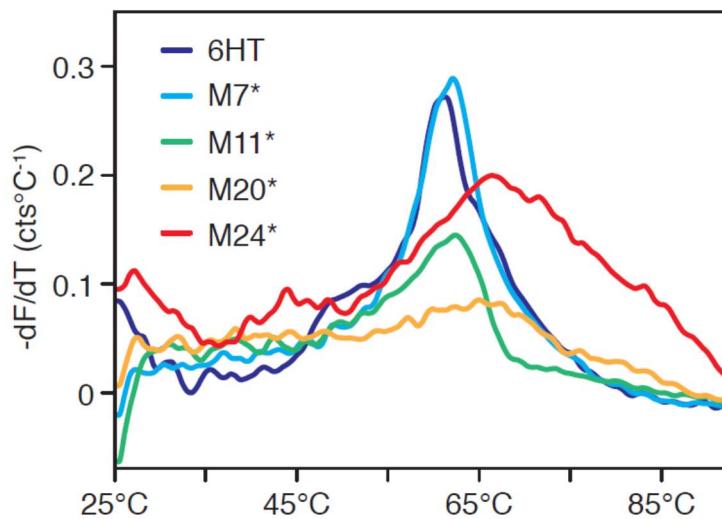


Figure S29. Melting profile diagram of 6HT, M7*, M11*, M20* and M24* tubes.

10. Thermal denaturing and subsequent Exo I digestion

This experiment was designed to mimic the denaturing conditions encountered in cells and other living systems, mainly due to low salt concentrations, in presence of an enzyme with nuclease activity. To this end, 6HT, M24 and M24* (1 pmol) were submitted to a heating step at 65°C for 15 minutes and then quickly cooled down in ice-water for 5 minutes. Afterwards the structures were submitted to Exo I digestion (10 U, 3 hours, 37°C) and then placed at -20°C to stop the enzymes activity. The samples were analyzed by 2% agarose gel containing 11mM MgCl₂ and TEM imaging (see figure 2b bottom, and 2c in the manuscript).

11. Incubation in Dulbecco's modified Eagle Medium (DMEM)

This experiment was designed to prove the stability of M24* tubes in a typical cell culture medium. To this end, 6HT, M24 and M24* (500 fmol) were diluted in DMEM (Merck Millipore) up to 12.5 µl. A negative control was made diluting 500 fmol of each structure in their folding buffer (1x TE Buffer, 20mM MgCl₂) up to 12.5 µl. Both the assay and the negative control were incubated 24 hours at 37°C in thermocycler. The samples were analyzed using a 2% agarose gel (0.5x TBE Buffer containing 11mM MgCl₂) (see figure 2b, top, in the manuscript).

12. Rate of strands incorporation in the structure and DNA-catenane formation yields

Strand incorporation rate and overall DNA-catenane formation yields were estimated to be almost quantitative by interpretation of various gels as shown in main text and in these pages. Anyway, agarose gel is surely not the preferred method to quantify the strand incorporation rate within the structure. It can be very challenging to verify if all 24 strands are incorporated into the structure and thus a 24-member DNA-catenane is formed after the click reaction merely by gel interpretation. The radioactive assay used by Ding et al.^[6] proved to be a reliable method to quantify strand incorporation rate, hence a fluorescent modified version of this assay was used in this work. Four *click*-tiles were selected among the 24 possible strands to be representative of a general behavior. Each of those *click*-tiles was fluorescent labelled with TAMRA and called hereinafter fluo-*click*-tiles. Four distinct tubes, each including one fluo-*click*-tile were separately folded. These tubes were loaded into agarose gels with or without MgCl₂ before and after click reaction and analyzed using a gel laser scanner. This experimental setup allows a better quantification of unfolded strands within the folded structure into the mixture. A schematic representation of this setup for one single tube is depicted in figures S30 and S31.

Agarose gel with $MgCl_2$

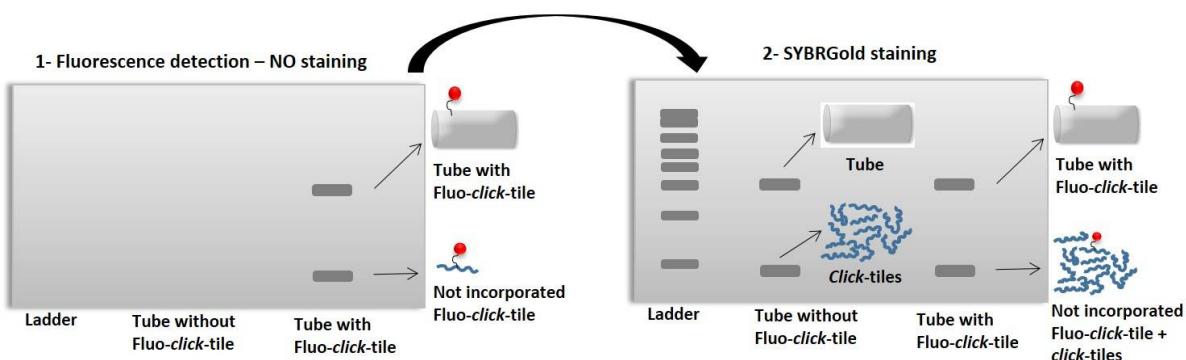


Figure S30. Schematic representation of the experimental setup for the strands incorporation rate test. Folded tubes containing one fluo-*click*-tile, along with other 23 *click*-tiles, are loaded into agarose gel containing $MgCl_2$. Gel is then imaged at gel laser scanner and only the samples containing fluo-*click*-tiles are detected (left gel picture). The same gel is afterwards stained with SYBRGold and hence even the structures without fluorescent labelling are visible (right gel picture).

Agarose gel without $MgCl_2$

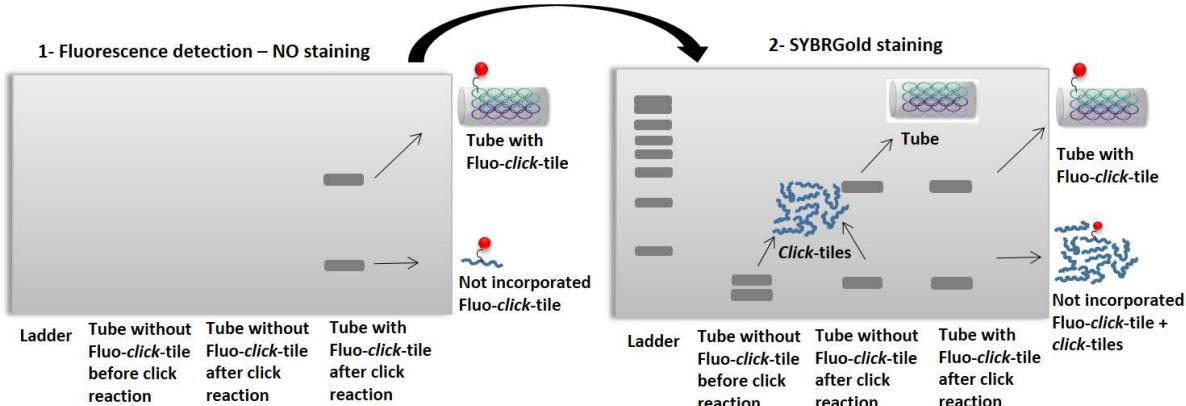


Figure S31. Schematic representation of the experimental setup for the rate of strands incorporation test. Tubes containing one fluo-*click*-tile, along with other 23 *click*-tiles, are loaded on agarose gel without $MgCl_2$. Gel is then imaged at gel laser scanner and only the samples containing fluo-*click*-tiles are detected. A low molecular weight band is to be expected only in case of incomplete click reaction (left gel picture). The same gel is afterwards stained with SYBRGold and hence even the structures without fluorescent labelling are visible. Only the structures submitted to click reaction will show the tube bands. Structures before click reaction will result in a band at low molecular weight (right gel picture).

Table S7. Sequences of the four TAMRA labelled *click-tiles* (fluo-*click-tiles*) used in this work. X and Z are the alkyne and the azide groups respectively as defined in figure S1. Y is dT-TAMRA.

Oligo code	Sequence	Length (bases)
V1C1	ZAAA ACGCTAAGCCA CCTTYAGATCC AAAX	28
V4C3	ZGCATAGACATT ATACYGTAAC ACCTTACGTA ACTTACAGCCX	42
V5C2	ZATTCYGAG AGAATTACGCC TATTCACATAG GCGAAGGCTX	42
V6C3	ZCCCATGATGCA AAGGGTCATG GGTCTTGAAA AATTAYGGCX	42

M24-A



Figure S32. Design of the completely modified tile assembled structure (all 24 strands are azide/alkyne modified) containing the fluo-*click-tile* V1C1 (M24-A). The red dot corresponds to the fluorescent labelled position.

M24-B



Figure S33. Design of the completely modified tile assembled structure (all 24 strands are azide/alkyne modified) containing the fluo-*click-tile* V4C3 (M24-B). The red dot corresponds to the fluorescent labelled position.

M24-C

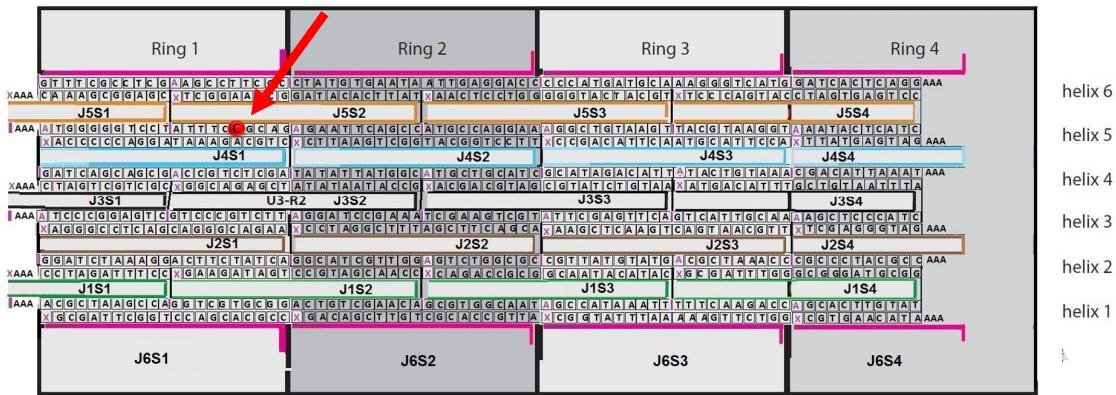


Figure S34. Design of the completely modified tile assembled structure (all 24 strands are azide/alkyne modified) containing the fluo-click-tile V5C2 (M24-C). The red dot corresponds to the fluorescent labelled position.

M24-D

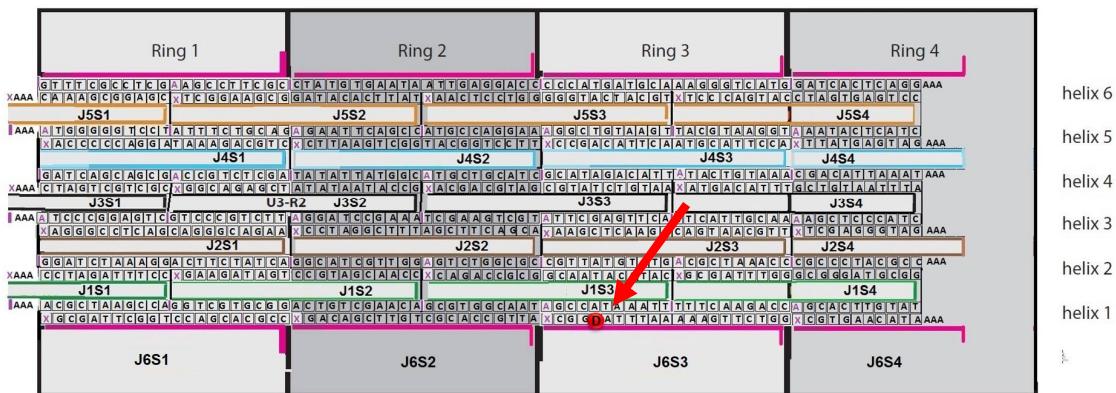


Figure S35. Design of the completely modified tile assembled structure(all 24 strands are azide/alkyne modified) containing the fluo-click-tile V6C3 (M24-D). The red dot corresponds to the fluorescent labelled position.

Tubes folding

Table S8. Schematic representation of strands composition of the different labelled tubes (M24A-D) used in the work. Each labelled strand (fluo-click-tile, highlighted by colored boxes) is added together with other 23 click-tiles.

M24-A				M24-B			
J5S1	J5S2	J5S3	J5S4	J5S1	J5S2	J5S3	J5S4
J4S1	J4S2	J4S3	J4S4	J4S1	J4S2	V4C3	J4S4
J3S1	J3S2	J3S3	J3S4	J3S1	J3S2	J3S3	J3S4
J2S1	J2S2	J2S3	J2S4	J2S1	J2S2	J2S3	J2S4
V1C1	J1S2	J1S3	J1S4	J1S1	J1S2	J1S3	J1S4
J6S1	J6S2	J6S3	J6S4	J6S1	J6S2	J6S3	J6S4
M24-C				M24-D			
J5S1	V5C2	J5S3	J5S4	J5S1	J5S2	J5S3	J5S4
J4S1	J4S2	J4S3	J4S4	J4S1	J4S2	J4S3	J4S4
J3S1	J3S2	J3S3	J3S4	J3S1	J3S2	J3S3	J3S4
J2S1	J2S2	J2S3	J2S4	J2S1	J2S2	J2S3	J2S4
J1S1	J1S2	J1S3	J1S4	J1S1	J1S2	J1S3	J1S4
J6S1	J6S2	J6S3	J6S4	J6S1	J6S2	V6C3	J6S4

Tubes are folded following the procedure and the folding program previously described (see page S9). After folding, tubes were reacted at 32°C for 5 hours with click reagents as described previously (see page S10).

Agarose gel containing MgCl₂ of folded fluorescent labelled tubes

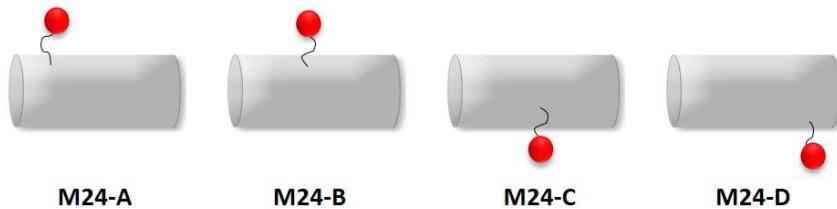


Figure S36. Schematic representation of M24-A, M24-B, M24-C and M24-D tubes (before click reaction).

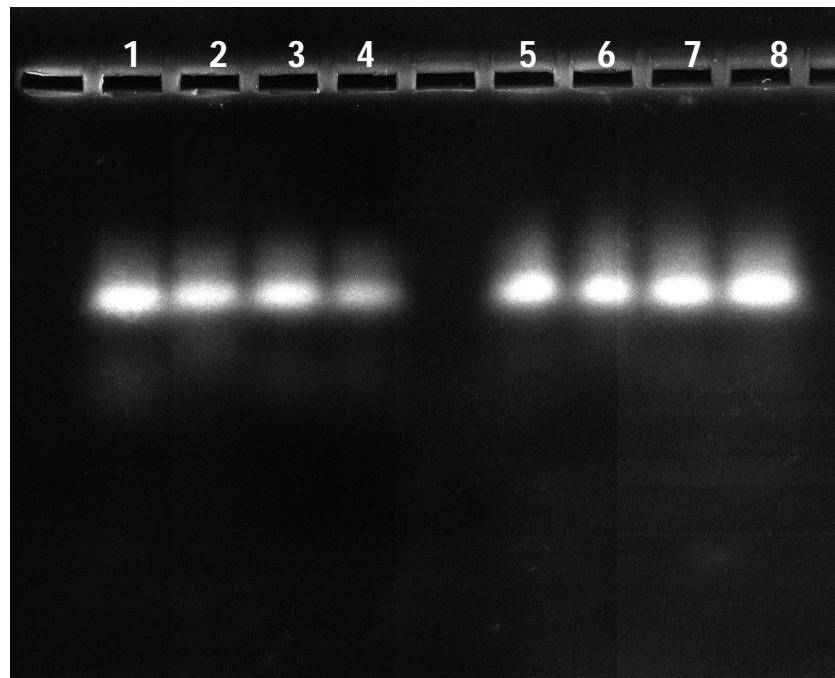


Figure S37. 2% agarose (0.5x TBE Buffer containing 11 mM MgCl₂), NO gel staining, only fluorescence detection. The fluorescence is due to TAMRA labelled strand incorporated into the structure. *Lane 1:* M24-A tube, no treatment; *lane 2:* M24-B tube, no treatment; *lane 3:* M24-C tube, no treatment; *lane 4:* M24-D tube, no treatment; *lane 5:* M24-A tube after Amicon purification; *lane 6:* M24-B tube after Amicon purification; *lane 7:* M24-C tube after Amicon purification; *lane 8:* M24-D tube after Amicon purification. The Amicon purification described in page S19 ensure removal of not incorporated strands from the mixture containing the tube.

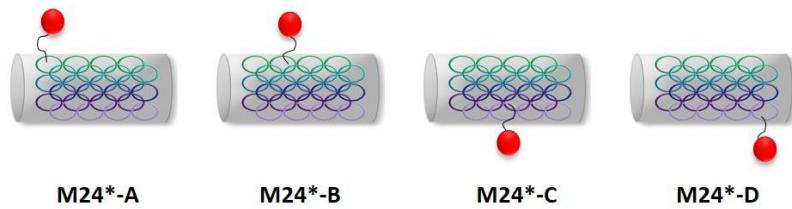


Figure S38. Schematic representation of M24*-A, M24*-B, M24*-C and M24*-D tubes (after click reaction).

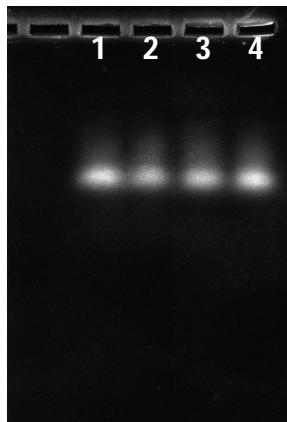


Figure S39. 2% agarose (0.5x TBE Buffer containing 11 mM MgCl₂), NO gel staining, only fluorescence detection. The fluorescence is due to TAMRA labelled strand incorporated into the structure. *Lane 1:* M24*-A tube; *lane 2:* M24*-B tube; *lane 3:* M24*-C tube; *lane 4:* M24*-D tube. Tubes have been submitted to click reaction after the Amicon purification step.

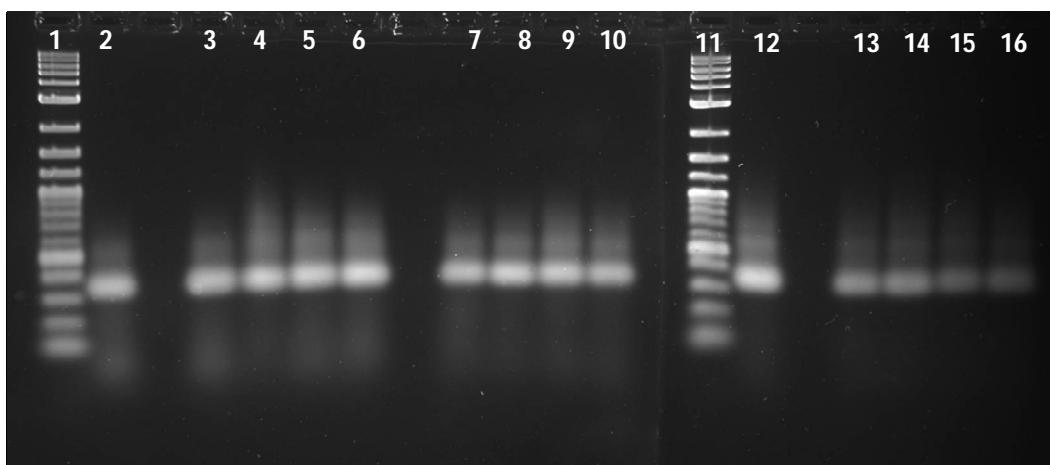


Figure S40. 2% agarose (0.5x TBE Buffer containing 11 mM MgCl₂), SYBRGold staining. *Lane 1:* 2-Log DNA ladder (New England Biolabs); *lane 2:* M24-tube, no treatment; *lane 3:* M24-A tube, no treatment; *lane 4:* M24-B tube, no treatment; *lane 5:* M24-C tube, no treatment; *lane 6:* M24-D tube, no treatment; *lane 7:* M24-A tube after Amicon purification; *lane 8:* M24-B tube after Amicon purification; *lane 9:* M24-C tube after Amicon purification; *lane 10:* M24-D tube after Amicon purification. *Lane 11:* 2-Log DNA ladder (New England Biolabs); *Lane 12:* M24*-X tube; *lane 13:* M24*-A tube; *lane 14:* M24*-B tube; *lane 15:* M24*-C tube; *lane 16:* M24*-D tube. The asterisk indicates that the tube was submitted to click reaction.

Agarose gel without MgCl₂ of fluorescent labelled tubes

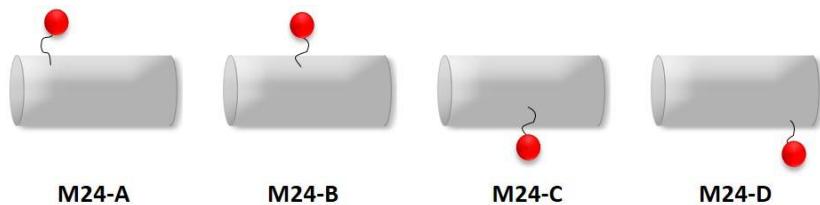


Figure S41. Schematic representation of M24-A, M24-B, M24-C and M24-D tubes (before click reaction).

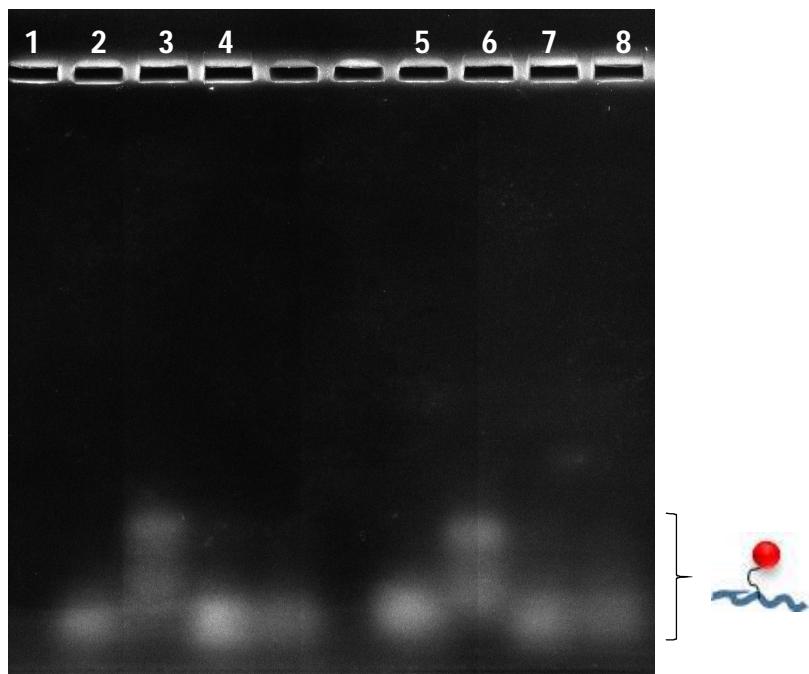


Figure S42. 2% agarose (0.5x TBE Buffer without MgCl₂), NO gel staining, only fluorescence detection. The fluorescence is due to TAMRA labelled strand. *Lane 1:* M24-A tube, no treatment; *lane 2:* M24-B tube, no treatment; *lane 3:* M24-C tube, no treatment; *lane 4:* M24-D tube, no treatment; *lane 5:* M24-A tube after Amicon purification; *lane 6:* M24-B tube after Amicon purification; *lane 7:* M24-C tube after Amicon purification; *lane 8:* M24-D tube after Amicon purification. Only the low molecular band of the TAMRA labelled strand is visible since the tube unfold during the run in the gel without MgCl₂.

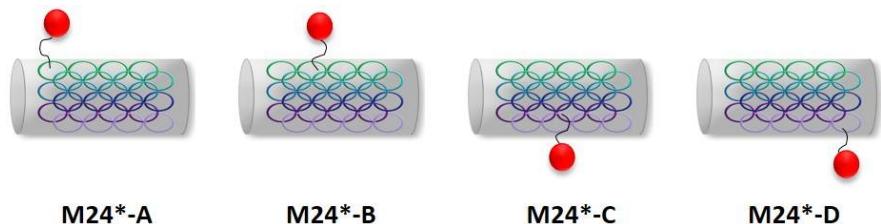


Figure S43. Schematic representation of M24*-A, M24*-B, M24*-C and M24*-D tubes (after click reaction).

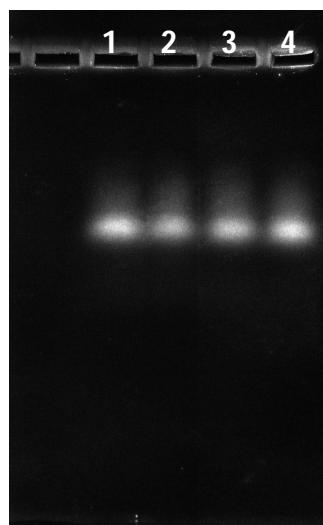


Figure S44. 2% agarose (0.5x TBE Buffer without MgCl₂), NO gel staining, only fluorescence detection. The fluorescence is due to TAMRA labelled strand incorporated into the structure. *Lane 1:* M24*-A tube; *lane 2:* M24*-B tube; *lane 3:* M24*-C tube; *lane 4:* M24*-D tube. Tubes have been submitted to click reaction after the Amicon purification step and remain folded also if loaded into gel without MgCl₂.

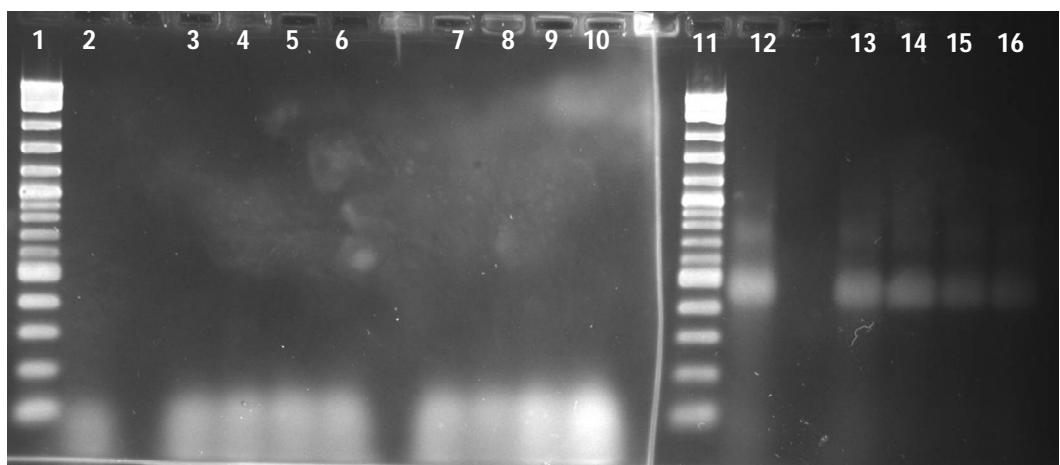


Figure S44. 2% agarose (0.5x TBE Buffer without MgCl₂), SYBRGold staining. *Lane 1:* 2-Log DNA ladder (New England Biolabs); *lane 2:* M24-tube, no treatment; *lane 3:* M24-A tube, no treatment; *lane 4:* M24-B tube, no treatment; *lane 5:* M24-C tube, no treatment; *lane 6:* M24-D tube, no treatment; *lane 7:* M24-A tube after Amicon purification; *lane 8:* M24-B tube after Amicon purification; *lane 9:* M24-C

tube after Amicon purification; *lane 10*: M24-D tube after Amicon purification. *Lane 11*: 2-Log DNA ladder (New England Biolabs); *Lane 12*: M24* tube; *lane 13*: M24*-A tube; *lane 14*: M24*-B tube; *lane 15*: M24*-C tube; *lane 16*: M24*-D tube. The asterisk indicates that the tube was submitted to click reaction.

Fluorescence plots from agarose gel containing MgCl₂.

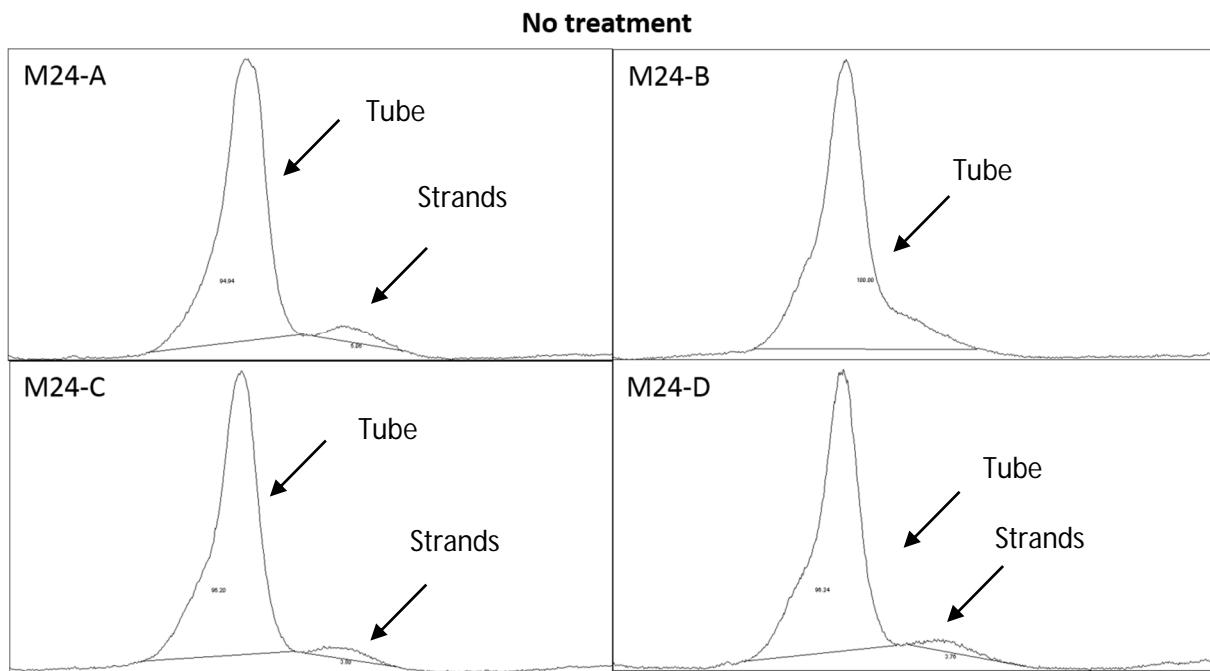


Figure S45. Fluorescence plots obtained from 2% agarose gel containing 11mM MgCl₂ (Figure S37 - M24-A tube = Lane 1; M24-B tube = Lane 2; M24-C tube = Lane 3; M24-D tube = Lane 4). Fluorescence detect for samples without treatment (after the folding). Percentages of fluorescence are reported in Table S9.

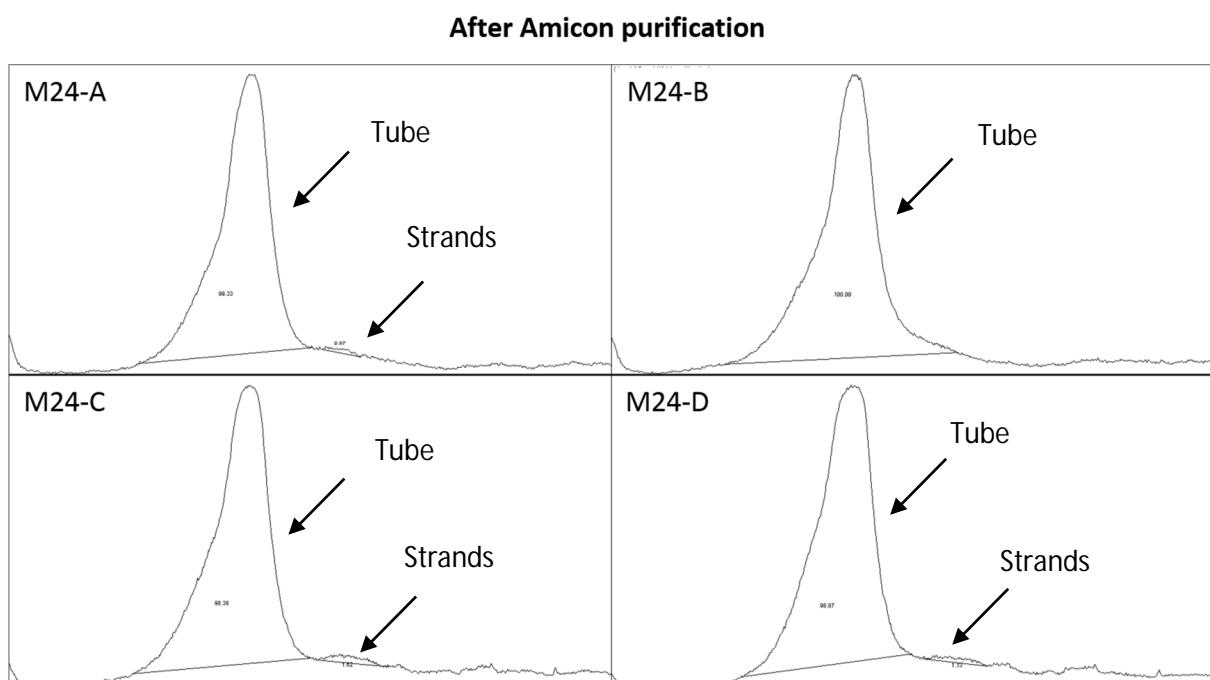


Figure S46. Fluorescence plots obtained from 2% agarose gel containing 11mM MgCl₂ (Figure S37 - M24-A tube = Lane 5; M24-B tube = Lane 6; M24-C tube = Lane 7; M24-D tube = Lane 8). Fluorescence detect for samples after Amicon purification. Percentages of fluorescence are reported in Table S9.

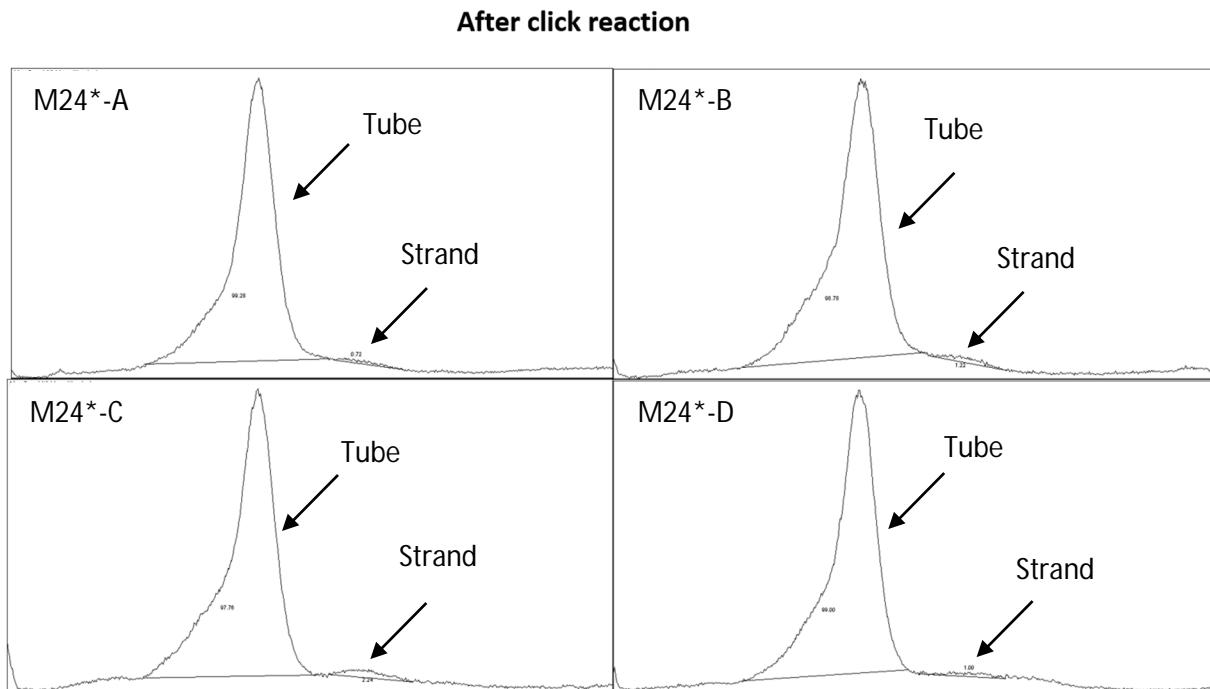


Figure S47. Fluorescence plots obtained from 2% agarose gel containing 11mM MgCl₂ (Figure S39 - M24-A tube = Lane 1; M24-B tube = Lane 2; M24-C tube = Lane 3; M24-D tube = Lane 4). Fluorescence detect for samples after click reaction. Percentages of fluorescence are reported in Table S9.

Table S9. Fluorescent bands quantification.

M24 A	% tube	% strands	M24 B	% tube	% strands
No treatment	94.9	5.1	No treatment	100.0	0.0
After Amicon	99.3	0.7	After Amicon	100.0	0.0
After click	99.3	0.7	After click	98.8	1.2

M24 C	% tube	% strands	M24 D	% tube	% strands
No treatment	96.2	3.8	No treatment	96.2	3.8
After Amicon	98.4	1.6	After Amicon	98.9	1.1
After click	97.8	2.2	After click	99.0	1.0

Fluorescence plots from agarose gel without MgCl₂.

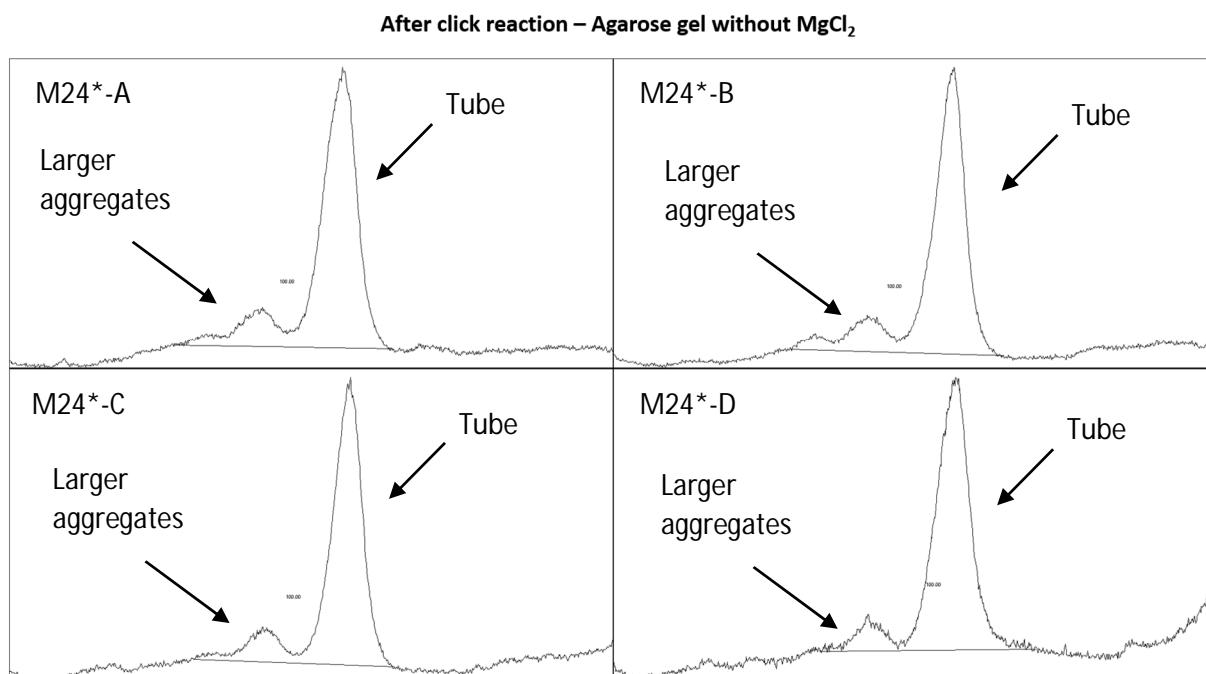


Figure S48. Fluorescence plots obtained from 2% agarose gel without MgCl₂ (Figure S44 - M24*-A tube = Lane 1; M24*-B tube = Lane 2; M24*-C tube = Lane 3; M24*-D tube = Lane 4). Fluorescence detection for samples after click reaction. No fluorescence was detected at low molecular weight, meaning that the click reaction has a quantitative yield.

In conclusion the detection of the fluorescent ratio between the folded tube and the low molecular weight band of the unfolded fluo-*click*-tiles showed a high efficient folding yield exceeding 95%. Moreover, using the same setup it was possible to quantify the efficacy of the click reaction, which showed quantitative yields for all four fluorescent tubes. This conclusion can be reasonably extended to all the other strands in the structure.

13. Catenation between terminal 28mers

The three short strands on one end of the structure (J1S1, J3S1 and J5S1, left side of Figure S3) have an extension of poly-dA (AAA) in both 3' and 5' ends, both functionalized with click reactive groups. In this case the alkyne and azide groups are not pre-assembled in that region and possible inter-strand reaction cannot be excluded.

In order to clarify this point a tube using 21 un-modified strands and those three short terminal *click*-tiles was folded. Additionally, a tube containing only one of those short terminal *click*-tiles was folded as control. The tubes were submitted to click reaction and then analyzed *via* denaturing PAGE. If inter-strand coupling occurs, then dimers or trimers of those *click*-tiles will be formed and thus detected in PAGE as higher molecular weight bands. If only intra-strand cyclization occurs, then only low molecular bands should be visible in the PAGE similarly to that generated by the control tube (see scheme in figure S49).

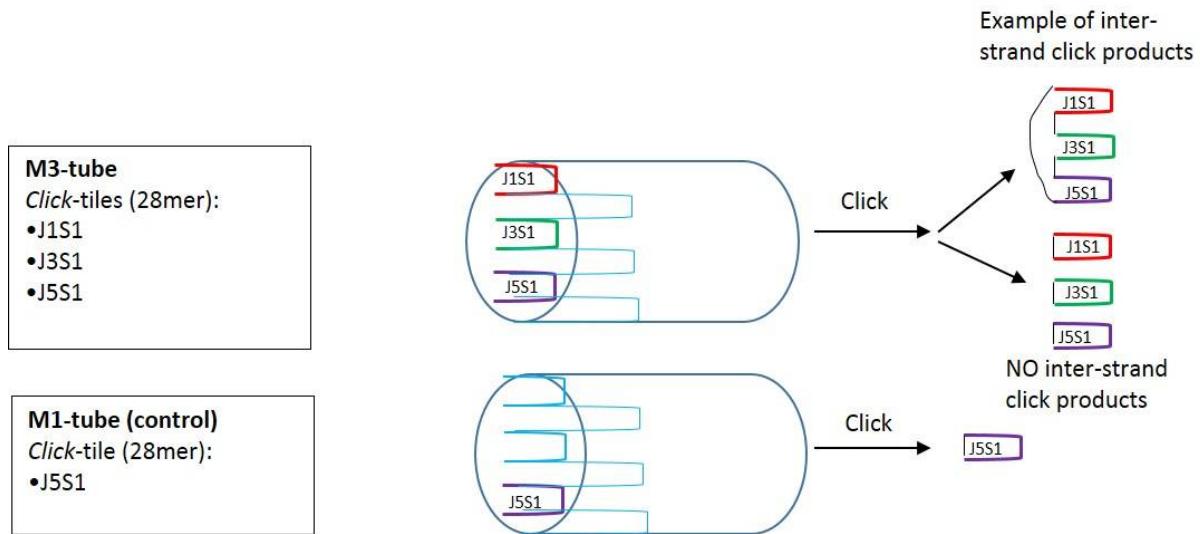


Figure S49. Scheme of the experimental setup to test possible inter-strand reactions among the terminal 28mer *click-tiles*.

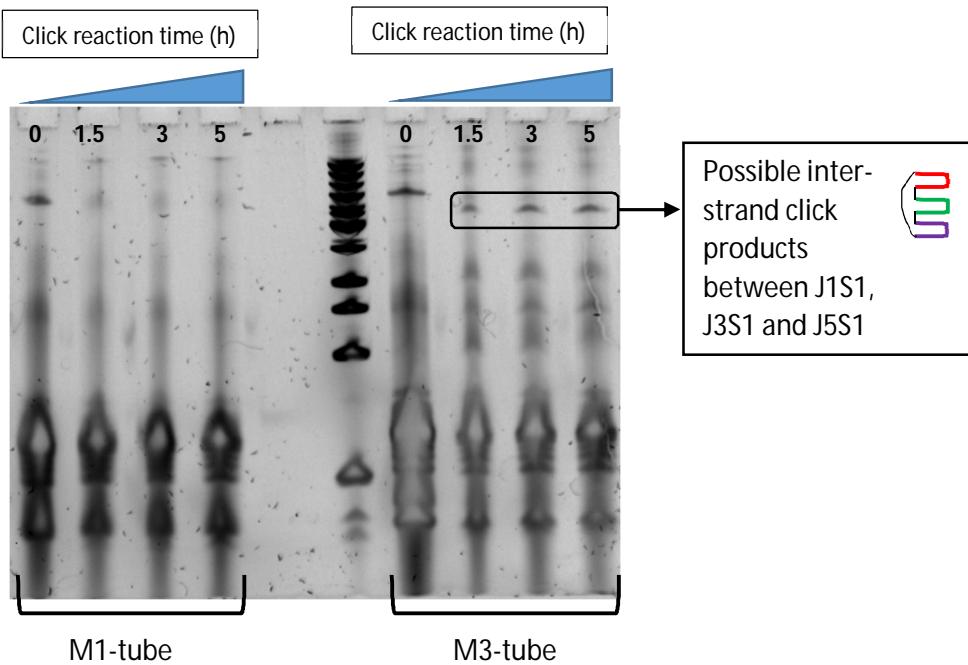


Figure S50. 10% denaturing PAGE of M1-tube and M3-tube, SYBRGold staining. From left to right: M1-tube before click reaction (lane 1), M1* tube submitted to click reaction for 1.5 hours (lane 2), for 3 hours (lane 3), for 5 hours (lane 4), Low molecular DNA ladder (New England Biolabs, lane 6), M3-tube before click reaction (lane 7), M3* tube submitted to click reaction for 1.5 hours (lane 8), for 3 hours (lane 9), for 5 hours (lane 10).

New bands are formed after click reaction of the M3-tube suggesting the formation of inter-strand structures. The bands increase in intensity with a longer reaction time. This “side-reaction” is due to the particular architecture of the tube containing poly-A ends and can be easily overcome with a proper design. The overall stability of this particular design is not affected by inter-strand reactions.

14. References

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