Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Chemical Ligation of an entire DNA Origami Nanostructure

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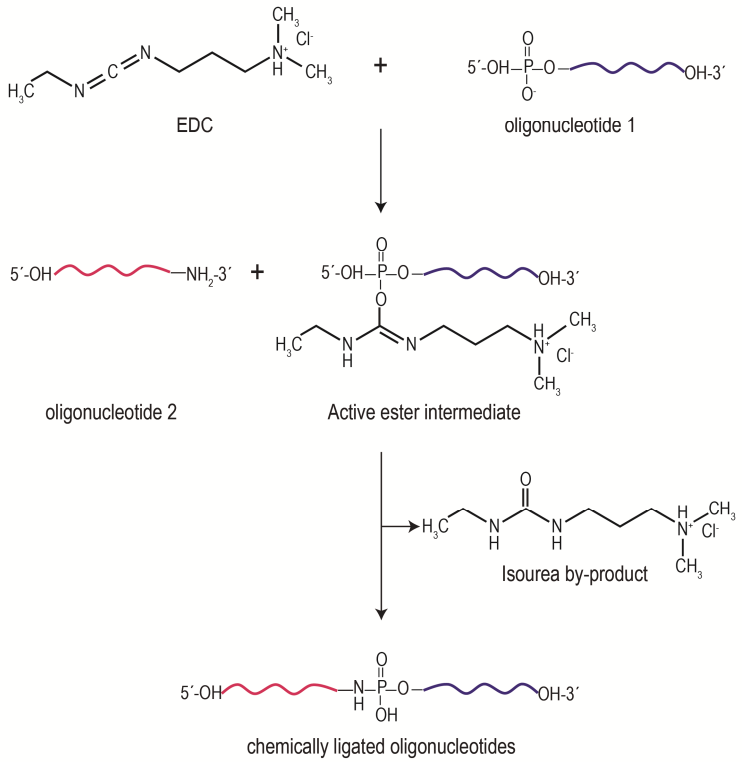

Figure S1. Scheme of the chemical ligation reaction, which consists of a chemoselective reaction of EDC to the 5' phosphate of an oligonucleotide 1 providing an active ester intermediate. The intermediate can be attacked by the 3' amine of an oligonucleotide 2 to create a covalent coupling between phosphate and amine which links the two oligonucleotides (adapted from Hermanson G. T., 2008).[1]

a)

O1-NH_2 (29 nt) + P-O2 (35 nt) + O3 (44 nt)

5′-GTTCAATCAGCACTATAATGTAATGGGA**T**AGGTCAGGTTGGTGTCGGACTCAGGAATATTTTTT-3′ (64 nt)
GTCGTGATATTACATTACCCTATCCAGTCCAACCACAGCCTGAG

b)

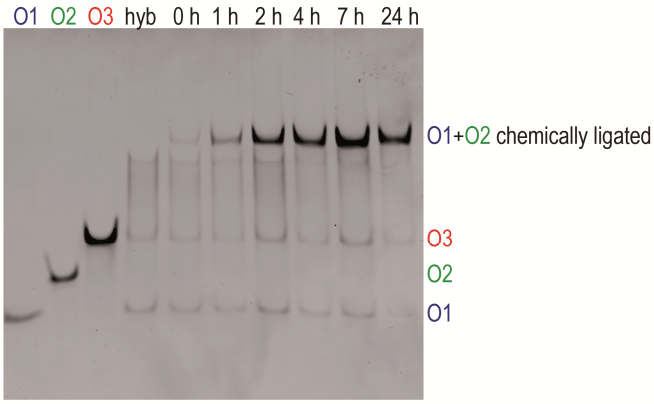

Figure S2. Time course of the chemical ligation reaction. a) Scheme of the employed DNA duplex with an internal nick subjected to ligation. The duplex consists of a 3' amine modified oligonucleotide O1 (in blue) and a 5' phosphorylated oligonucleotide O2 (in green), which are hybridized to a carrier oligonucleotide O3 (in red). The amine modification of O1 was accomplished by enzymatically adding the terminal thymidine (shown in bold) using 3'-Amino-2',3'-ddTTP. b) Denaturing PAGE (15 %) analysis of O1, O2 and O3, of the hybrid of the three oligonucleotides (hyb) and of the hybrid after varying time points (shown above) of the chemical ligation reaction.

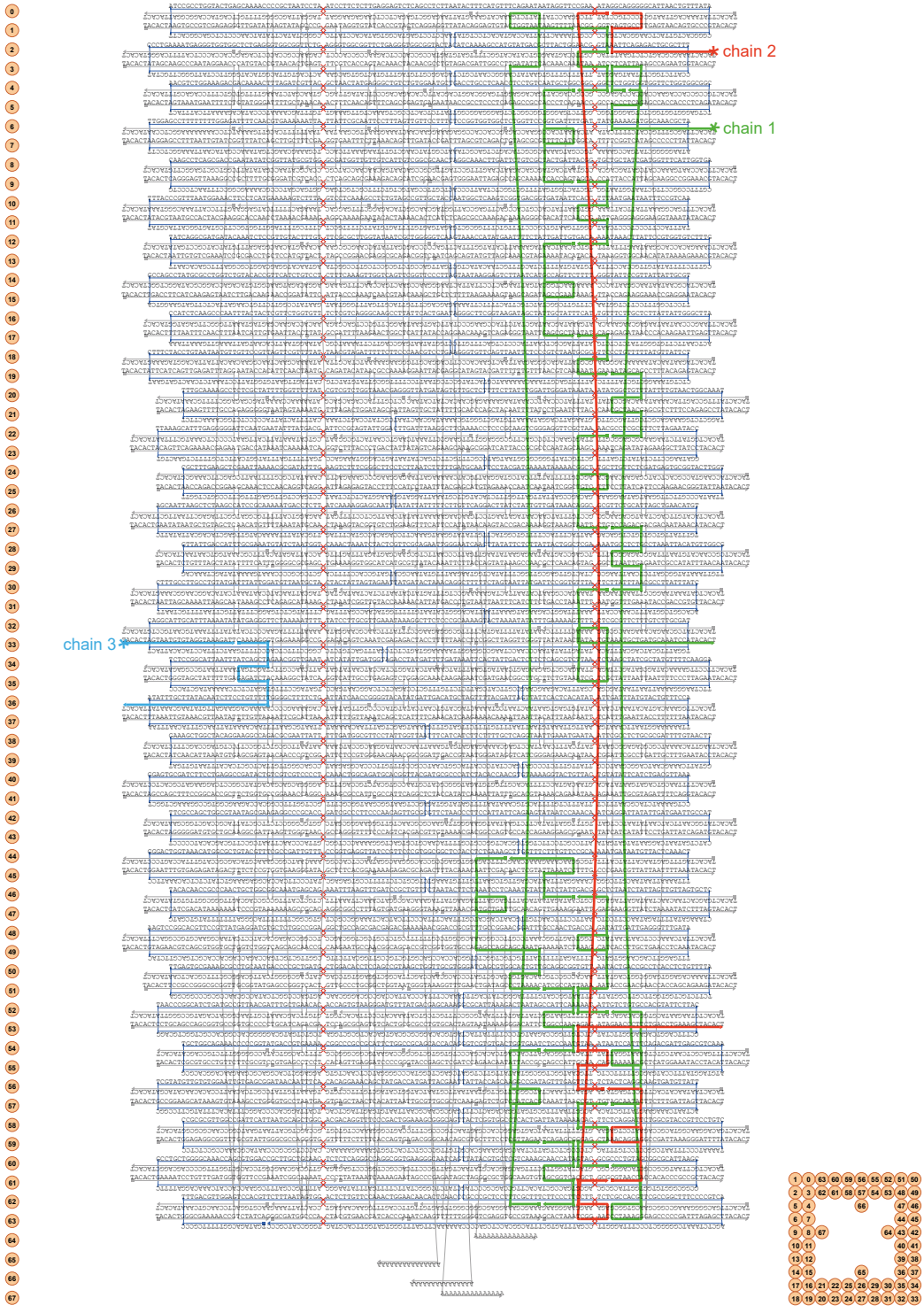

Figure S3. Design template of the DNA origami tube structure with highlighted set 1 staple chains. Shown are the 8064 nt scaffold (blue) and the different staples (other colors) including their sequences. Staples of the ligation chains 1, 2 and 3 (set 1) are shown in green, red and light blue, respectively (Table S1). Labels of the first staple of a particular ligation chain are marked with an aterisk.

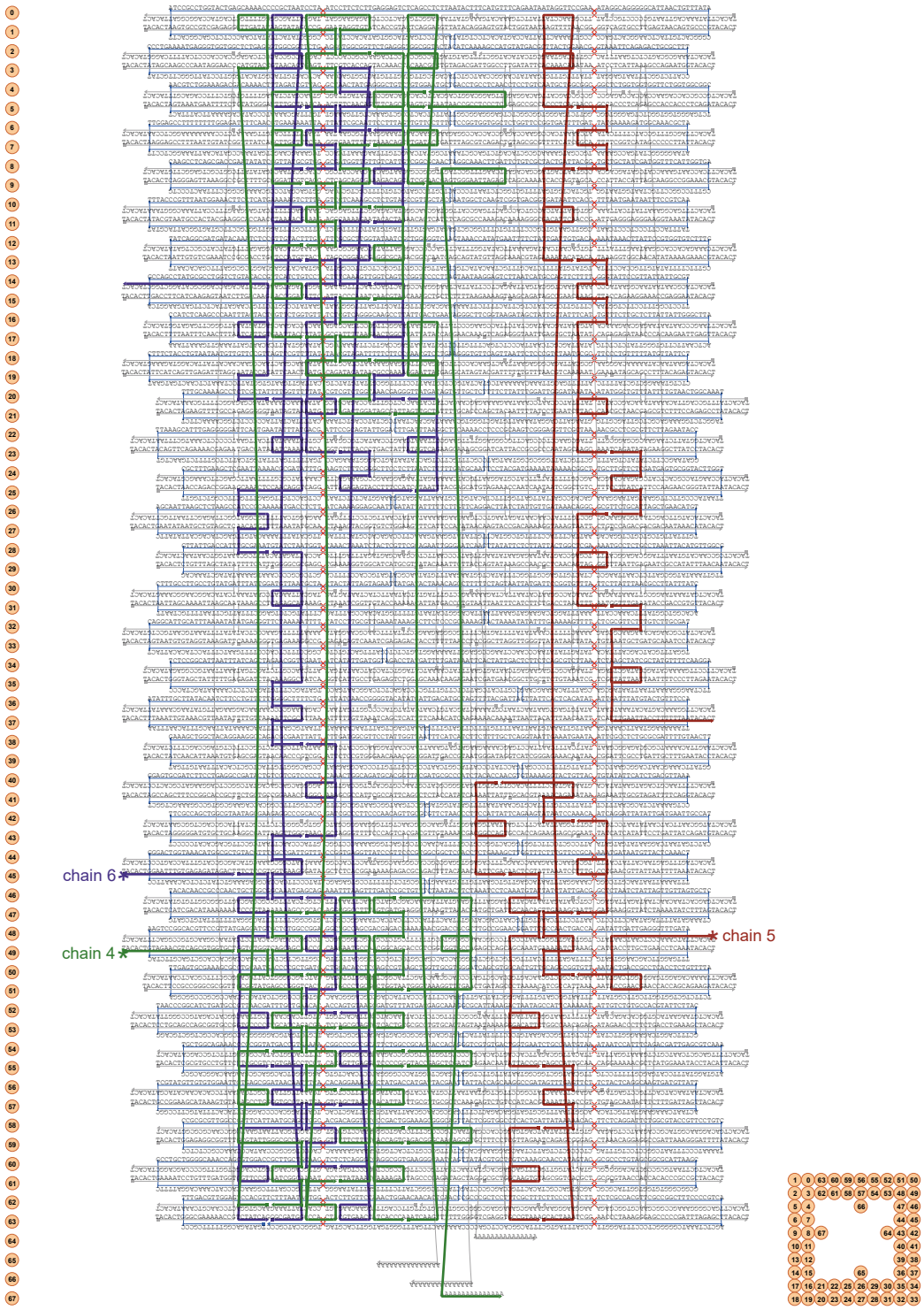

Figure S4. Design template of the DNA origami tube structure with highlighted set 2 staple chains. Shown are the 8064 nt scaffold (blue) and the different staples (other colors) including their sequences. Staples of the ligation chains 4, 5 and 6 (set 2) are shown in dark green, dark red and dark purple, respectively (Table S2). Labels of the first staple of a particular ligation chain are marked with an aterisk.

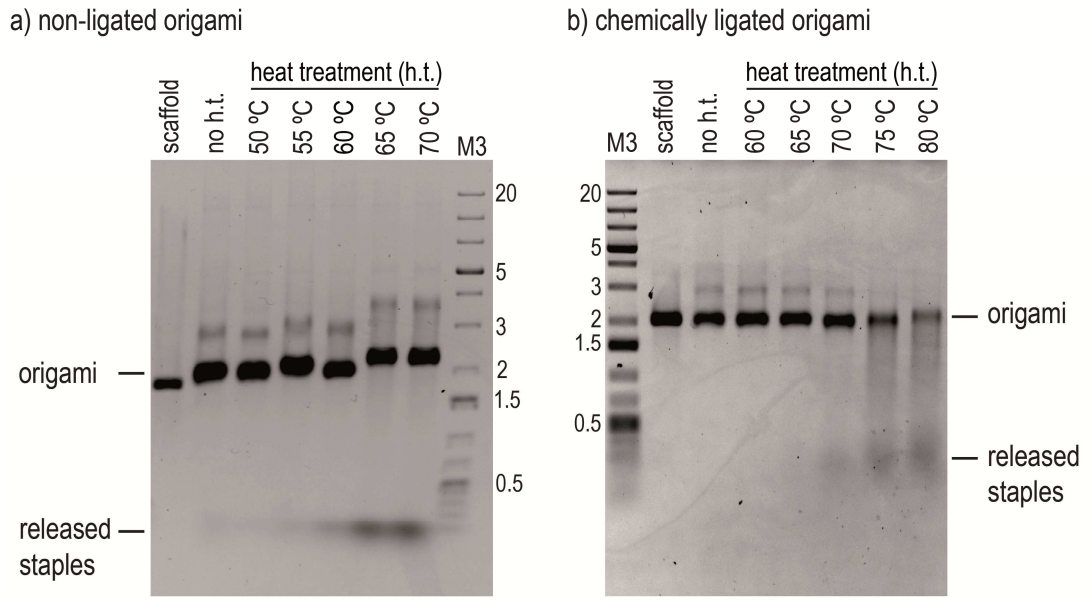

Figure S5. Analysis of the heat stability of non-ligated and chemically ligated DNA origami nanostructures using agarose gel electrophoresis (1 %). a) Analysis of the non-ligated DNA origami nanostructures before and after incubation for 10 min at the indicated temperatures. b) Analysis of the chemically ligated DNA origami nanostructures before and after incubation for 10 min at the indicated temperatures. Numbers next to bands of the DNA size marker (M3) indicate the corresponding DNA lengths.

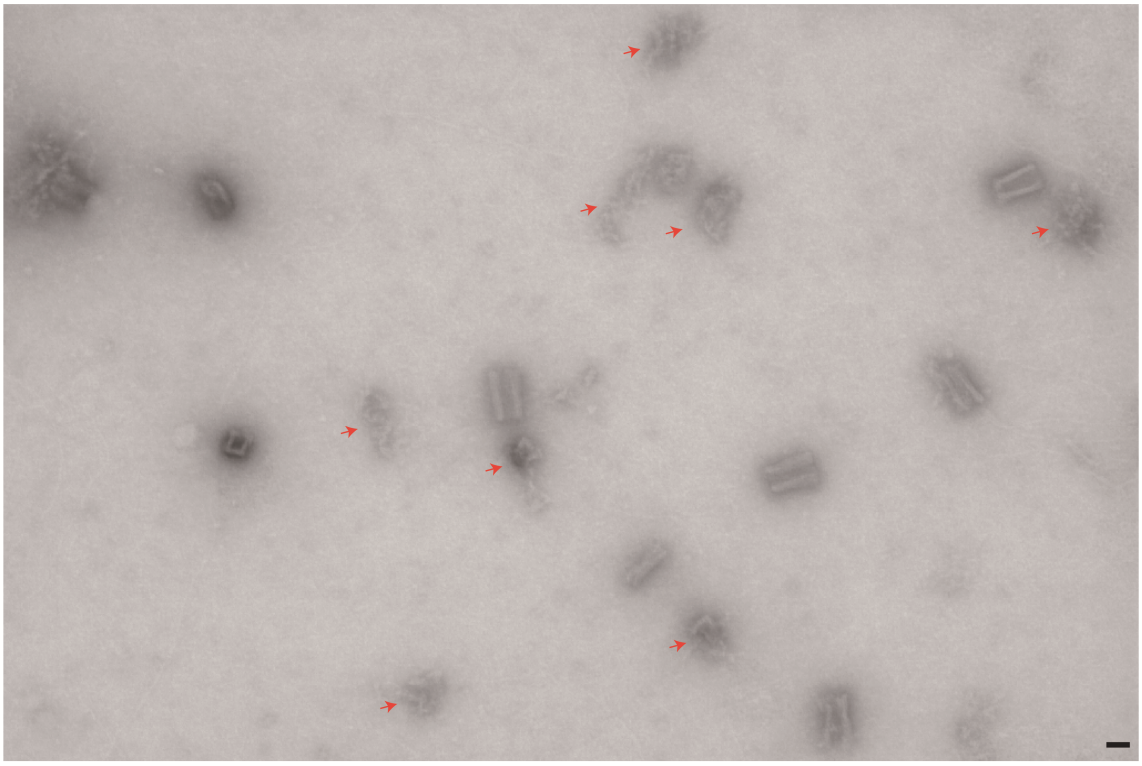

Figure S6. Overview tSEM image of non-ligated DNA origami nanostructures incubated for 10 min at 60 °C. Red arrows indicate disordered structures, i.e. origami nanostructures that already disassembled (Figure 3, main text). The scale bar corresponds to 20 mm.

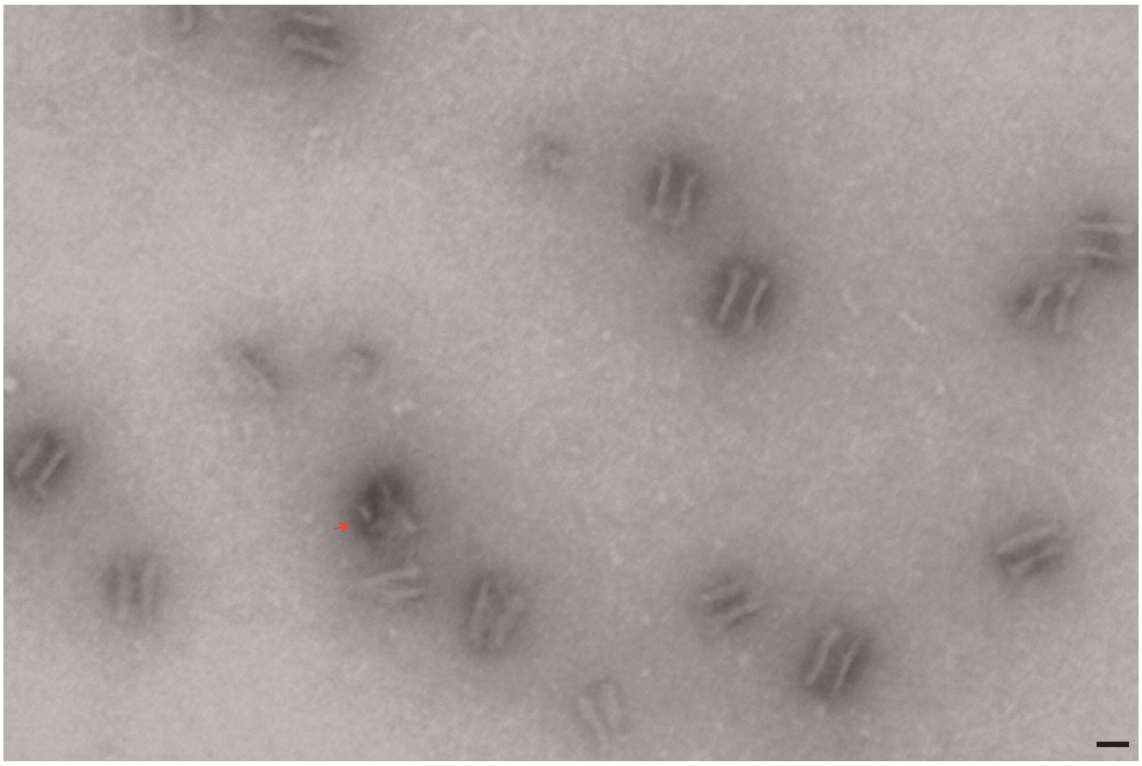

Figure S7. Overview tSEM image of the chemically ligated DNA origami nanostructures after incubation at 70 °C for 10 min. Red arrows indicate disordered structures, i.e. origami nanostructures that already disassembled (Figure 3, main text). The scale bar corresponds to 20 mm.

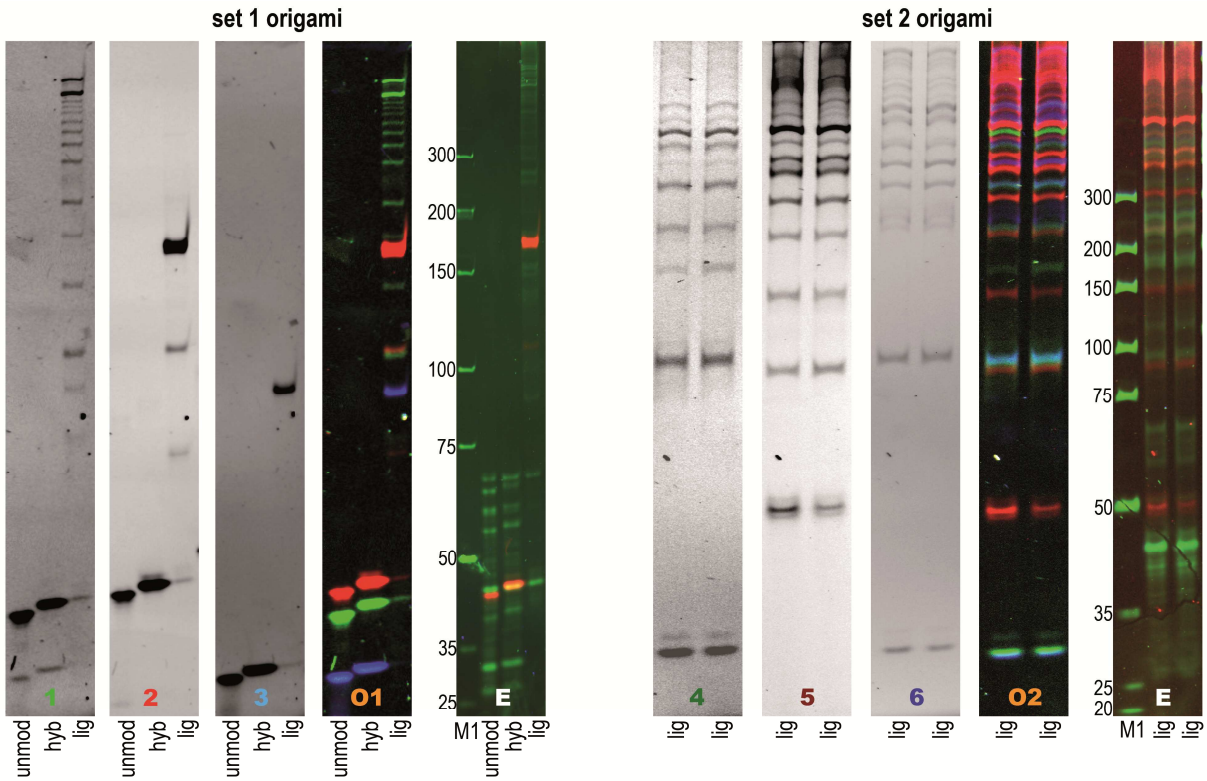

Figure S8. Enlarged views on the denaturing PAGE (8 %) analysis of the chain ligation (Figure 4, main text). Using different fluorescent labels for the first staple of a chain, chain ligations were probed in parallel in a single origami structure for chains 1-3 (set 1 origami, left side) as well as for chains 4-6 (set 2 origami, right side, see also main text incl. Figure 4 for further details). Gel images marked with numbers show the fluorescence emission of the first staple of the particular chain (Cy3 emission for chains 1 and 4, Cy5 emission for chains 2 and 5 and 6-FAM emission for chains 3 and 6). Gel images marked with O1 and O2 show false colour overlays of the emissions the chain starts of the particular origami structure. Gel images marked with E show the EtBr stained total DNA and the Cy5 emissions. Lane labels correspond to unmodified staples (unmod), non-ligated origami nanostructures (hyb) and chemically ligated origami structures (lig). Numbers next to the bands of the DNA size marker (M1) indicate the corresponding DNA lengths.

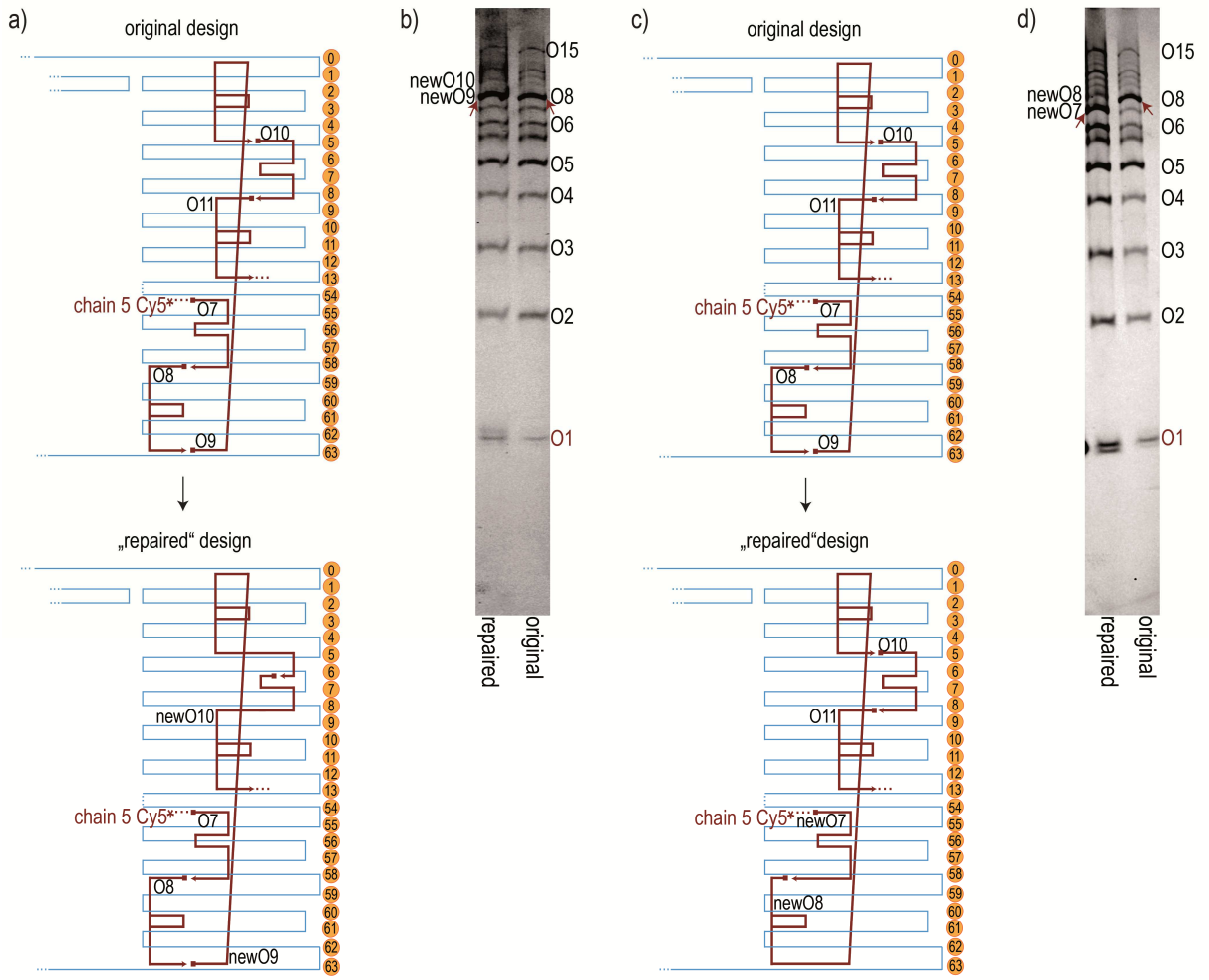

Figure S9. Identifying and ‘repairing’ design problems within an origami structure using chain ligation. (a) Enlarged view on the origami design containing oligonucleotides 9, 10 and 11 of chain 5 from set 2 origami (Figure S4 and Table S2) before (top) and after (bottom) the intended design repair. In the altered design two new staples (newO9 and newO10) replaced the original staples 9, 10 and 11 (Table S3). Helix numbers of the design are shown on the right. b) Denaturing PAGE (8 %) analysis of the set 2 origami monitoring the terminal Cy5 label of chain 5 before and after the design repair (Figure 4, Figure S8). The chain ligation terminates with for the original design with high probability at staple 8 (see red arrow in right lane) indicating an assembly problem at this position. The altered design did not increase the ligation efficiency at this position (red arrow in left lane), such that the ‘repair’ was unsuccessful. c) Enlarged view on the origami design containing oligonucleotides 7, 8 and 9 of chain 5 from set 2 origami (Figure S4, and Table S2) before (top) and after (bottom) another design repair. For this design alteration the new staples newO7 and newO8 replaced the original staples 7, 8 and 9 (Table S3). Helix numbers of the design are shown on the right. d) Denaturing PAGE (8 %) analysis of chain 5 of set 2 origami before and after the design repair with newO7 and newO8 (Figure 4, Figure S8). Also in this case the inefficient ligation at step 8 could not be alleviated (see red arrows in both lanes).

Table S1. Ligation chain details of the set 1 origami (Figure 4, main text and Figure S3). Lengths of the individual staples and resulting chain length for a successful chain ligation including the given staple. The staple number (No.) provides the order of the staple with respect to the 5' end of the chain.

	chain 1 (Cy3)		chain 2 (Cy5)		chain 3 (6-FAM)	
No.	Staple length	length of chain	Staple length	length of chain	Staple length	length of chain
1	37 nt[a)]	37 nt	41 nt[a)]	41 nt	27 nt[a)]	27 nt
2	36 nt	73 nt	28 nt	69 nt	59 nt	86 nt
3	28 nt	101 nt	33 nt	102 nt		
4	32 nt	133 nt	54 nt	156 nt		
5	35 nt	168 nt				
6	32 nt	200 nt				
7	56 nt	256 nt				
8	32 nt	288 nt				
9	32 nt	320 nt				
10	32 nt	352 nt				
11	40 nt	392 nt				
12	28 nt	420 nt				
13	32 nt	452 nt				
14	28 nt	480 nt				
15	32 nt	512 nt				
16	21 nt	533 nt				
17	32 nt	565 nt				
18	28 nt	593 nt				
19	32 nt	625 nt				
20	53 nt	678 nt				

a) Indicates the first staples of a chain that carries the indicated fluorescent label on its 5' end

Table S2. Ligation chain details of the set 2 origami (Figure 4, main text and Figure S4). Lengths of the individual staples and resulting chain length for a successful chain ligation including the given staple. The staple number (No.) provides the order of the staple with respect to the 5' end of the chain.

	chain 4 (Cy3)		chain 5 (Cy5)		chain 6 (6-FAM)	
No.	Staple length	length of chain	Staple length	length of chain	Staple length	length of chain
1	27 nt[a)]	27 nt	48 nt[a)]	48 nt	27 nt[a)]	27 nt
2	56 nt	83 nt	32 nt	80 nt	56 nt	83 nt
3	64 nt	147 nt	28 nt	108 nt	64 nt	147 nt
4	40 nt	187 nt	34 nt	142 nt	40 nt	187 nt
5	60 nt	247 nt	30 nt	172 nt	28 nt	215 nt
6	28 nt	275 nt	56 nt	228 nt	32 nt	247 nt
7	32 nt	307 nt	32 nt	260 nt	49 nt	296 nt
8	50 nt	357 nt	32 nt	292 nt	34 nt	330 nt
9	43 nt	400 nt	40 nt	332 nt	30 nt	360 nt
10	32 nt	432 nt	28 nt	360 nt	40 nt	400 nt
11	32 nt	464 nt	32 nt	392 nt	32 nt	432 nt
12	32 nt	496 nt	49 nt	441 nt	32 nt	464 nt
13	32 nt	528 nt	32 nt	473 nt	56 nt	520 nt
14	52 nt	580 nt	28 nt	501 nt	28 nt	548 nt
15	32 nt	612 nt	57 nt	558 nt	32 nt	580 nt
16	28 nt	640 nt			35 nt	615 nt
17	39 nt	679 nt			32 nt	647 nt
18	32 nt	711 nt			28 nt	675 nt
19	31 nt	742 nt			56 nt	731 nt
20	24 nt	766 nt			64 nt	795 nt
21	50 nt	816 nt			67 nt	862 nt
22	46 nt	862 nt				
23	32 nt	894 nt				
24	32 nt	926 nt				
25	32 nt	958 nt				
26	32 nt	990 nt				
27	37 nt	1,027 nt				
28	38 nt	1,065 nt				
29	26 nt	1,091 nt				
30	50 nt	1,141 nt				

a) Indicates the first staples of a chain that carries the indicated fluorescent label on its 5' end

Table S3. Sequences of the staples that were changed upon design 'repairs' (Figure 5 and Figure S9) as well as the sequences of the staples of the new design. For the altered designs, three successive original staple sequences were fused and split into two new staple sequences.

chain 1 of set 1 origami (Figure 5)

Staple	Sequence (5' to 3')	nt
O15	ACAATCAAAGCCGAACGAACTGGCGAGCGCTA	32
O16	ATATCAGGAAGCGCAAATGAA	21
O17	AATAGCAGAACAGCCACGCTAACGCGCGAGGC	32
newO15	ACAATCAAAGCCGAACGAACTGGCGAGCGCTAATATCAGGAA	42
newO16	GCGCAAATGAAAATAGCAGAACAGCCACGCTAACGCGCGAGGC	43

chain 5 of set 2 origami (Figure S9)

Staple	Sequence (5' to 3')	nt
O7	CAGCCATTCTCAAACTCAAATTAATTTTATAA	32
O8	TCAGTGAGGCAAGTGTCTTTGACGGCCGTAAA	32
O9	GCACTAAAGAACCTATACAAACAACCAGTAAGACCCTCAG	40
newO7	CAGCCATTCTCAAACTCAAATTAATTTTATAATCAGT	37
newO8	GAGGCAAGTGTCTTTGACGGCCGTAAAGCACTAAAGAACCTATACAAACAA-CCAGTAAGACCCTCAG	67
O9	GCACTAAAGAACCTATACAAACAACCAGTAAGACCCTCAG	40
O10	AACCGCCATAATCAGGCATTTGCACCGT	28
O11	AATCAGTAGACATTCAAATTATCAAAAATACA	32
newO9	GCACTAAAGAACCTATACAAACAACCAGTAAGACCCTCAGAACCGCCATA	50
newO10	ATCAGGCATTTGCACCGTAATCAGTAGACATTCAAATTATCAAAAATACA	50

References

1 G. T. Hermanson, *Bioconjugate Techniques. Second Edition*, Academic Press, Cambridge, MA, 2008.