Electronic Supplementary Information

for

manuscript entitled

M1.3 - A Small Scaffold for DNA Origami

by

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1. Materials and Methods

Materials

Single-stranded M13mp18 was purchased from *Bayou Biolabs* (Materie, LA, USA), from which M1.3 scaffold was excised as described in the Experimental Part of the main paper. For the 4F origami sheet, cartridge-purified staple oligonucleotides were purchased from *Biomers* (Ulm, Germany) and were used without modification. ATTO-labeled Oligonucleotides were obtained from Eurofins MWG/Operon in HPLC-purified form and were used further purification. Concentrations of M1.3 scaffold and oligonucleotide solutions were determined by measuring the UV absorbance with a *Nanodrop ND-100* spectrophotometer (*NanoDrop Technologies*, Willmington, DE, USA). Restriction enzymes were purchased from *Fermentas* (St. Leon Rot, Germany). For DNA extraction from agarose gels, a *NucleoSpin gel and PCR clean-up* kit (Macherey-Nagel, Düren, Germany) was used. Buffer salts and agarose were obtained from *Carl Roth* (Karlsruhe, Germany). Amicon Ultra filter devices (MWCO 30,000 Da) were purchased from *Millipore* (Billerica, MA, USA). TEM grids were from Plano (Wetzlar, Germany). The 0.24-9.49 kb RNA ladder for gels was purchased from *Life Technologies* (Darmstadt, Germany). The 100 bp ladder was received from *New England Biolabs* (Ipswich, MA, USA).

TEM Imaging

For analysis of the four finger sheet (4F sheet), 0.5 pmol of linear M1.3 was allowed to assemble using 10 eq of the staple strands, as described in the Experimental Part of the main paper. The sample was diluted with folding buffer (5 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 12 mM $MgCl_2$) to give a 1 nM solution of the scaffold. Transmission electron microscopy employed a sample of the solution (3 µL) that was applied to a carbon-coated glow-discharged TEM grid. After 1 min, excess liquid was soaked up with filter paper, and the sample was stained with 1% uranyl acetate for 30 s. The grid was dried in air, and the sample was visualized at 68000-fold magnification with a *FEI Tecnai* G^2 transmission electron microscope.

For electron microscopy of the other origamis, a similar procedure was employed, using argon plasma-cleaned grid, placing 1.5 µL of gel purified sample for 1 min on the grids, washing with 7 µL uranyl acetate (1%), and staining with 7 µL uranyl acetate (1%) for 8 s.

2. Full Sequence of M1.3 scaffold (704 nt, linear version)

5’-

AATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGCACTGGCCGTCGTTTTA
CAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGC
GTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCT
GGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCC
CCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTGACCTATCCCATTACGGTCAATCCGCC
GTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGC
CAGACGCGAATTATTTTTGATGGCGTTCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAATGCGAATTTT
AACAAAATATTAACGTTTACAATTTAAATATTTGCTTATACAATCTTCCTGTTTTTGGGGCTTTTCTGATTATCAACC
GGGGTACATATGATTGACATGCTAGTTTTACGATTACCGTTCATCGATTCTCTTGTTTGCTCCAGACTCTCAGGCAA
TGACCTGATAGCCTTTGTA

-3‘

3. Other restriction enzyme combinations for excising fragments from single-stranded M13mp18 DNA

3.1 Overview of other successful excision reactions

a HindIII + BsrBI: restriction fragment size of 708 nucleotides

b HindIII + BglII: restriction fragment size of 653 nucleotides

c EcoRI + BglII: restriction fragment size of 704 nucleotides

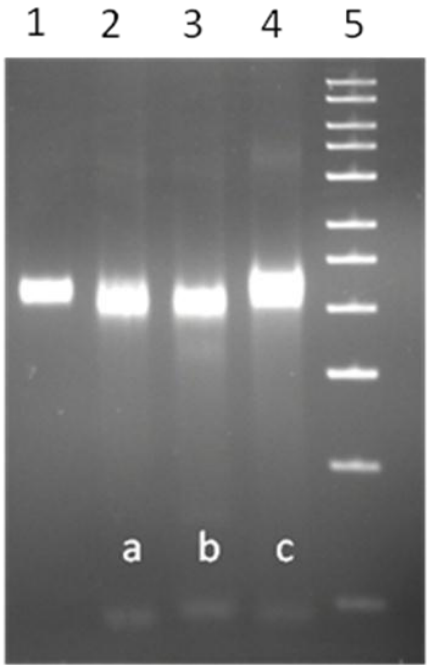


Fig. S1. Excising M1.3 with other enzyme combinations.
Lane 1: 0.2 pmol M13; *Lane 2*: 0.4 pmol M13 after digestion with HindIII and BsrBI; *Lane 3*: 0.4 pmol M13 after digestion with HindIII and BglII; *Lane 4*: 0.4 pmol M13 after digestion with EcoRI and BglII; *Lane 5*: Lambda DNA/PstI digest ladder.

3.2 The special case of excision with NaeI + Eco53kI: restriction fragment size of 624 nucleotides

- NaeI recognition site: 5'…GCCGGC…3'
- Eco53kI recognition site: 5'…GAGCTC…3'

Initially, with these two enzymes, no restriction fragment was detected in agarose gels. NaeI is a type II restriction enzyme, for which both cleavable and resistant NaeI recognition sequences were found.[1] In fact, NaeI is a type IIe subclass restriction enzyme, requiring the recognition of a second DNA site to induce cleavage. Hence, some DNA sequences, including M13mp18, with a single recognition site, cannot be readily cleaved by NaeI. Topal and coworkers found that the cleavage of resistant sites can be induced by adding appropriate external DNA duplexes.[1] By adding a DNA duplex that contains a NaeI recognition site, a successful cleavage of single-stranded M13mp18 DNA was achieved. The sequence of this duplex is:

5'-TGG TGG GCG CCG GCG GTG TGG GCA-3'
3'-ACC ACC CGC GGC CGC CAC ACC CGT-5'

Shown below is the gel after the cleavage reaction.

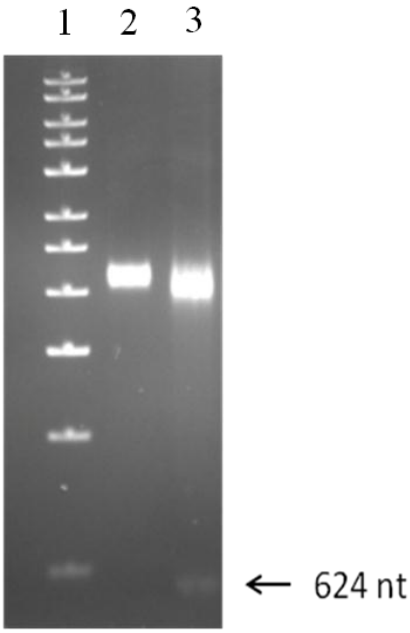
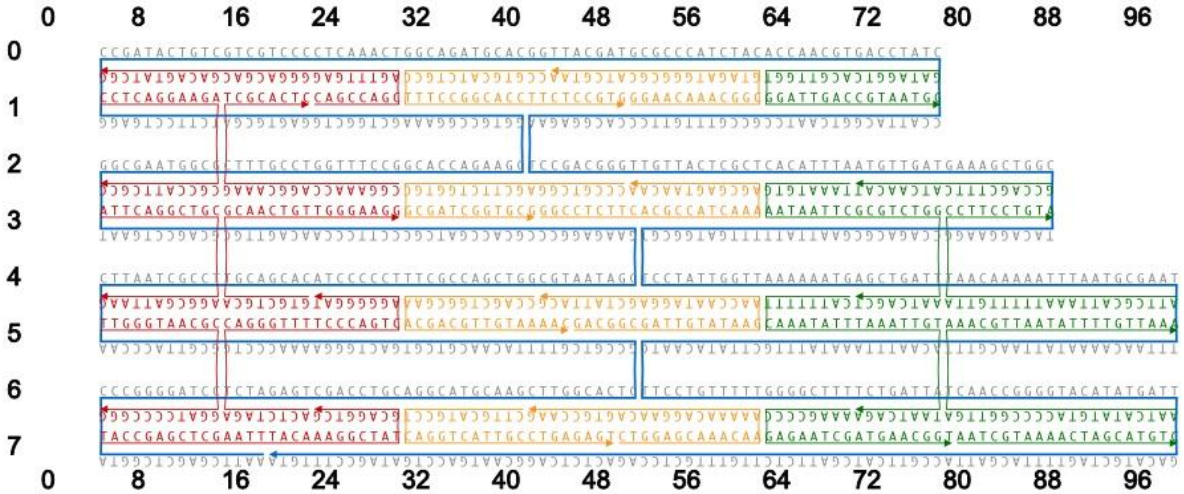


Fig. S2. Excising M1.3 with restriction enzymes NaeI and Eco53kI.
Lane 1: 0.5 µg Lambda DNA/PstI digest ladder; *Lane 2*: 0.2 pmol M13; *Lane 3*: 0.4 pmol M13 after digestion with Eco53kI and NaeI.

4. Origami Designs

Four Finger Sheet (compare Figure 2)



Staple strands sequences

Start	End	Sequence 5'→3'	Length
0[79]	1[79]	GATAGGTCACGTTGGTGGATTGACCGTAATGG	32
2[71]	4[72]	TAAATGTGAATAATTCGCGTCTGGAATCAGCT	32
2[89]	2[72]	GCCAGCTTTCATCAACAT	18
4[71]	6[72]	CATTTTTTCAAATATTTAAATTGTTAATCAGA	32
4[100]	3[89]	ATTCGCATTAAATTTTTGTTACCTTCCTGTA	31
6[71]	7[80]	AAAGCCCCGAGAATCGATGAACGGT	25
6[100]	5[100]	AATCATATGTACCCCGGTTGAAAACGTTAATATTTTGTTAAA	42
7[81]	7[100]	AATCGTAAAACTAGCATGTC	20
1[5]	2[5]	CCTCAGGAAGACGCCATTCGCC	22
1[24]	0[5]	CAGCCAGCAGTTTGAGGGGACGACGACAGTATCGG	35
2[31]	1[23]	CGGAAACCAGGCAAAGTCGCACTC	24
3[5]	4[5]	ATTCAGGCTGCAGGCGATTAAG	22
4[23]	3[31]	GTGCTGCAGCAACTGTTGGGAAGG	24

5[5]	6[5]	TTGGGTAACGCGGATCCCCGGG	22
6[23]	4[24]	ACTCTAGACAGGGTTTTCCCAGTCAGGGGGAT	32
7[5]	6[24]	TACCGAGCTCGAATTTACAAAGGCTATGCAGGTCG	35
0[44]	1[51]	CCGTGCATCTGCCTTTCCGGCACCTTCTCCGTG	33
1[52]	0[45]	GGAACAAACGGCGTAGATGGGCGCATCGTAA	31
2[51]	3[43]	CCCGTCGGAGCTTCTGGTGCGCGATCGGTGCG	32
3[44]	2[52]	GGCCTCTTCACGCCATCAAAAGCGAGTAACAA	32
4[43]	5[46]	CCAGCTGGCGAAACGACGTTGTAAAAC	27
5[47]	4[44]	GACGGCGATTGTATAAGAACCAATAGGAGCTATTACG	37
6[42]	7[50]	CTTGCATGCCTCAGGTCATTGCCTGAGAGT	30
7[51]	6[43]	CTGGAGCAAACAAAAAAACAGGAACAGTGCCAAG	34

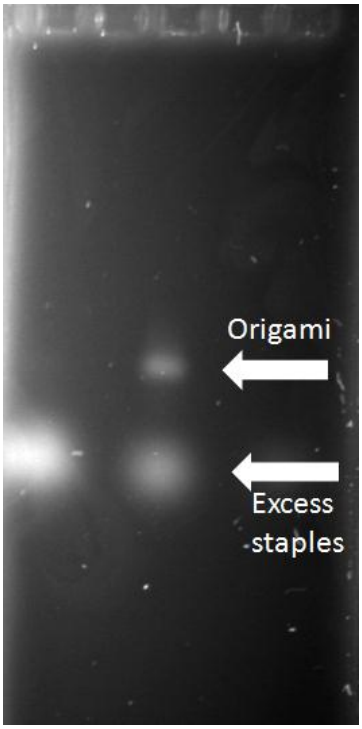
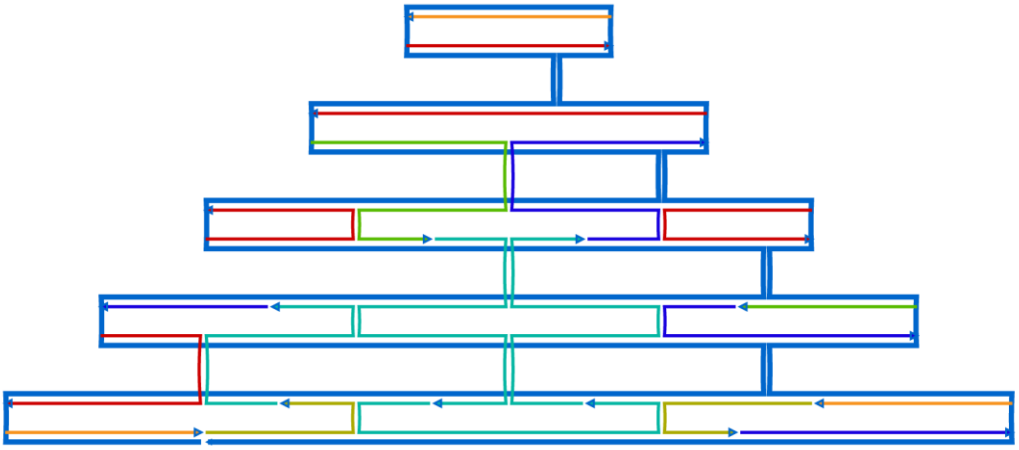


Fig. S3. Additional Agarose gel of four finger M1.3 sheet with ethidium bromide staining. For a different gel of the same assembly, see Figure 2 of the main paper. Left-most lane: M1.3 at high concentration, without staples; center lane: four finger origami sheet assembled with 10 equivalents of staple strands.

Other M1.3 Origami Designs (compare Figure 3)

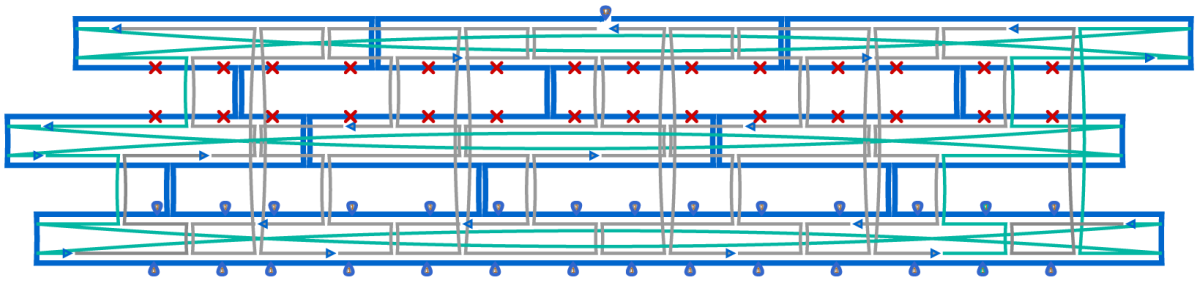
2D Triangle (square lattice)



2D Triangle

GCATGCCTCGCTATTACGCCAGCTGCGCAACTG
AAGATCGCATTCGCCATTCAGGCTGGCGAAAGGGGGATGTAAAACGAC
GGCCAGTGCAATCATATGTACCCCGGTTGATAATCAGAAACCAGTCAC
GACGTTGTGCTGCAAGGCGATTAAAACCAGGCAAAGCGCCACTCCAGC
TGTTACCAGGGTTTTCAGCCCCAA
CGTAAAACTAGCATGTCCAAGCTT
TTGGGAAGGGCGATCGGTTTTT
AAACAGGAAGATTGTATAAGCAAATATTTTTTT
GTGCCGGAGTTGGGTAACGAAATTCGCATTAAATTTTTT
CAGCTTTCGTTGGTGTAGATGGGCTAATGGGATAGGTCACCCATCTTTT
TTTTTTTGTTAAATCAGCTCCTG
TTTTGAACAAACGGCGGATTGACCGGCATCGTAACCGTGCAGCCTCAGG
TTTTCATTAAATGTGAGCGATGGCCTTTTT
TTTTTTAACCAATAGGAACGCGGCACCGCTTATTTTTTTT
TTTTGACGACGACAGTATCGTCTGCCAGTTTGAGGGTTTT
TTTTGCGGGCCTCTTGCAGGTCGACTCTAGAGGATCTTTT
TTTTAAAAATAATTCGCGTCGTAACAACCCGTCGGATTCTCCGTGGTTTT
TCCTGTAGCTCCTCTTTTGAGGAACAAGTTTTCTTGTCAGCTTTCATCAA
AAATTGTATCCTCTTTTGAGGAACAAGTTTTCTTGTAACGTTAATATTT
CCCGGGTTCCTCTTTTGAGGAACAAGTTTTCTTGTACCGAGCTCGAATT

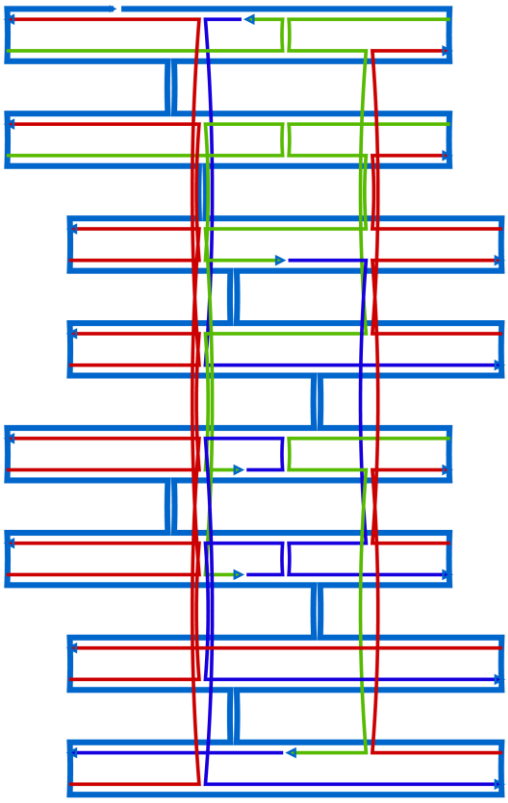
Curved 6 helix bundle with deletions (red crosses) and insertions (blue loops) (honeycomb lattice)



curved 6 helix bundle

AGGTCATCGGTCGGCAGATGAACGGTAGTAAAGACGTTCC
AGGCTGCCCCTTCACCGTCTTCGCTTGCCTCATATCGGCAAG
GAGAATTGCCTGATTCTCCGTGGGAACAACCAGTCACACGACGG
TGCGGGGCAACTGTTGGTGTA
ACTAAATAAAGCAGCAAAGCCCC
TGGTTGCCAAGCCGTAACGCCAGGGTTTTCACGGCGGACGAATT
TGTATTTCATTAGCTTAAATTGTTATTTTGGTGAGCGAG
CCAGTGCATGCCTACTCTAGAGGATCCGAGCTTTGACCGT
AAAAACAGCATTAAATTTAAAATTCGCATTA
CCGGTTGACCGTCGGAGAGTCTGGAGTTAAAAACGCTAAA
GCGATTAGGTCACGTTGGGAAGGGGGTACCCCGCCCAGC
TAACAACTAATCAGAATGTCAATCATATGTAATCGAAACAA
AATGGGATAAGTTGGGAAAGGGGGATGTGCATTACGGATCGG
AGTTTGAGGGGACCATCGTAAGCCATTC
CAGCTTTCATCAAGACGTCTGATTCGAGAT

3D Cube (square lattice)



3D Cube

ATGGGGAGCGAGTAACAACCCTTTT
CGCTATTAACTCCAGCGCGTATCGGCCTCAGGTTTT
CGAGTATTTAAATTGCCATTCAGGCTGCGCAACTTTT
TCTGACGTTAATTGGGAACAAACGGCGGATTGACCGTAATGTTTT
ATTCCAGCTTTCTGTCGCATCGTAACCGTGCATCTGCCAGTTTTT
TTTTAGCAAACAAGAGAATCGCCAAGCTTGCGTAC
TTTTAAAGCGCCATTCGTAAGTGCCGGATGGTGTAG
TTTTAAAACTAGCATGTCAATCATGGGTAACCGACGGCCAGCCTCTT
TTTTTCTAGAGGATCCCCGGATGCCTGCGATCGGTGCGGGCCCCCGCT
TTTTTCACGACGTTGTAAAAGCCAGGGTTGCAAGGCGATTAAGTAATA
TTTTAAGGGGGATGTGCTTTCCCAGTTTT
TTTTTGTTGGGAAGGGCAGGTCGACTTTT
TTTTAAGATCGCCGCCAGCTGGCGATTTT
TTTTGGATAGGTCACGTAACCAGGCTTTT
TTTTTGATAATCAGAAAAGTGATGAACGGTAATCGTTTTT
TTTTTTGTATAAGCAAACTCGAATTTGAGAGTCTGGTTTT
TTTTTAAATTTTTGTTAAATCACAAAAACAGGAAGATTTT
TTTTCAATAGGAACGCCATCAAATATGTACCCCGGTTTTT
TTTTCATCAACATTAAACGGCAGCTCATTTTTTAACTTTT
TTTTGTCGGATTCTCCGATTTTGTTAAAATTCGCATTTTT
TTTTTTGAGGGGACGACGACAGTCTGGCCTTCCTGTAGCCAGCTTTTTTT

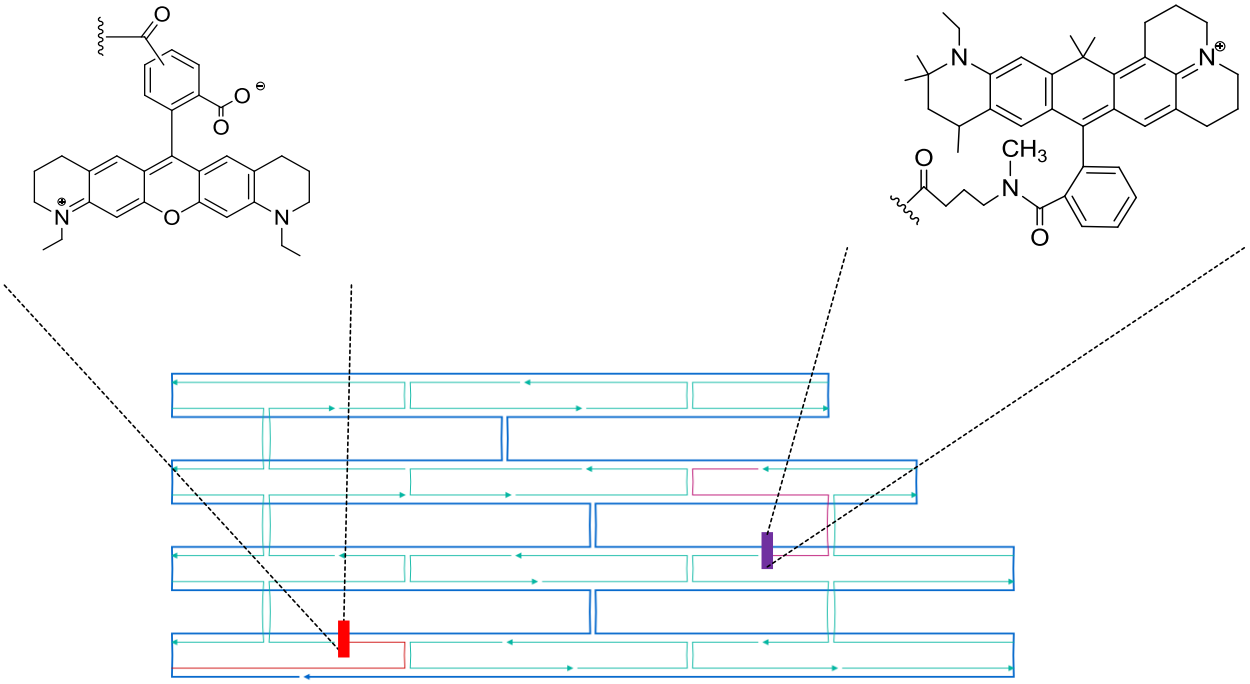
5. ALEX data, stoichiometry histogram for fluorescently labeled pair of staples

5.1 Design of 4F M1.3 sheet with two fluorophore-labeled oligonucleotides

Sequences of labeled oligonucleotides

Start	End	Sequence 5'→3'-Terminus	Length
7[5]	6[24]	TACCGAGCTCGAATTTACAAAGGCTATGCAGGTCGTTT – Atto565	35
2[71]	4[72]	TAAATGTGAATAATTCGCGTCTGGAATCAGCTTTT– Atto647N	32

Structure of doubly labeled 4F Sheet and position of fluorophores (Atto565 is shown in red and Atto647N is shown in purple, all other staple sequences unchanged)



5.2 Sample Preparation, Data Acquisition and Data Evaluation.

Annealed 4F sheets were purified using Amicon Ultra-0.5mL centrifugal filters (100,000 MW cut-off) 4× at 14'000g for 5 min. Between each centrifugal step, 500 µL of buffer (TE with 12

mM $MgCl_2$) were added. After the last step, the filter was turned and spun at 1000 g for 1 min to recover the sample. The single-molecule fluorescence measurements were carried out at 21°C in standard phosphate buffer saline (PBS) with Trolox to reduce blinking and bleaching.[3] To prevent DNA accumulation at the surface, the sample chambers were incubated with BSA (1 mg/ml BSA in PBS) prior to the measurements. The ALEX measurements were performed on a custom-built confocal microscope that is described in detail in reference 4. The light intensities were 20 µW at 533 nm for Atto565 and 20 µW at 640 nm for Atto647N. Data evaluation was performed as described in reference 4. The parameters of the Seidel burst search algorithm for the 4F sheet were T=0.5 ms, M=30, L=50. The dye molecules are alternately excited and the fluorescence of Atto565 and Atto647N is detected separately. The stoichiometry value S is defined by the Atto565 emission induced by excitation at 533 nm $F^{Atto565}$ and the Atto647N emission induced by excitation at 640 nm $F^{Atto647N}$ (reference 5).

$$S = F^{Atto565} / (F^{Atto565} + F^{Atto647N})$$

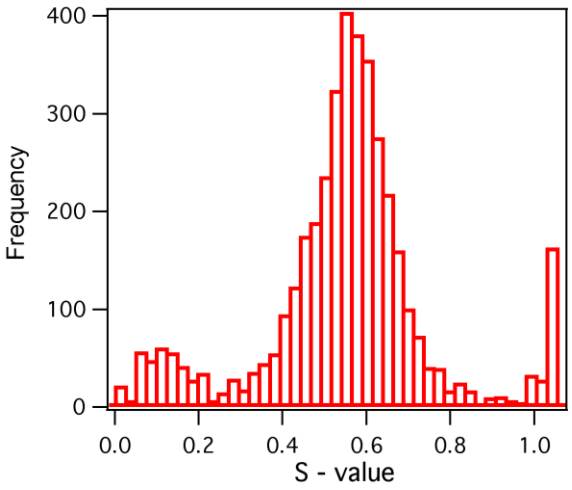


Fig. S4. Stoichiometry histogram of fluorescently labeled 4F sheet that describes the ratio between the dyes Atto565 and Atto647N. A large fraction of 4F sheets has integrated both fluorescently labeled staple strands (S = 0.5). Due to the burst search algorithm that identifies doubly labeled structures preferentially over singly labeled molecules, the absolute ratio between doubly labeled and singly labeled 4F sheets cannot be determined.

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6. Estimated cost of producing 1 mg of M13 single-stranded DNA scaffold by conventional molecular biology methodology

Estimated cost of materials required for mid-scale production of M13 circular single-stranded DNA in a German university molecular biology setting (excluding costs for equipment, energy, and labor). Similar costs will arise when using one-way purification cartridges for single-stranded DNA, which may speed up the procedure by one to two days.

Bio/Chemicals for the preparation of 1 l phage-multiplying E. coli culture (expected yield of M13 single-stranded DNA: 0.1-1 mg/l)

	€ prices
LB broth medium (1 l)	
Trypton 10 g	1.20 €
NaCl 10 g	0.02
Yeast Extract 5 g	1.20
Reagents (biology quality)	
$MgCl_2$	0.05
PEG-6000 or similar	0.80
NaCl	0.12
Tris buffer, pH adjusted	0.08
Phenol, buffer-saturated	12.00
Chloroform	1.70
Na-acetate pH 4.8	0.40
Ethanol (Rotisol)	1.20
Ultrapure water, buffer, etc	0.20
antibiotics, agar etc.	
Consumables approx.	1.00
Quality control approx.	2.00

(agarose, ethidium bromide, loading/electrophoresis buffer, documentation)

Total **22.00** (not including VAT)

Time effort: approx. 3-4 days during a full week, as specified below
(1 person working, including preparation, sterilization, clean-up etc.):

Day 1: Preparation of media and equipment, plating of starter culture: ca. 5 h
Day 2: Set-up of liquid pre-cultures and transfer into cold room: ca. 1 h
Day 3: Set-up of 10 production cultures à 100 ml (works better than one large culture) and infection by M13: approx. 2 h (including pre-treatment of shaker etc.), plus harvest: 2 h
Day 4: Isolation, including DNA precipitation
Day 5: DNA resuspension and quality control *via* gel electrophoresis; documentation.

7. Gel of 4F M1.3 Origami with 5'-Capped Staples

The formation of M1.3 with the 5'-pyrenyl capped staple strands was analyzed by using agarose gel electrophoresis. In both cases, with the **PyC** and the **PyPy** cap, a clear band was detected. As shown below, the bands of the modified M1.3 origami gave a modest shift in the gel in comparison with the unmodified 4F M1.3 origami.

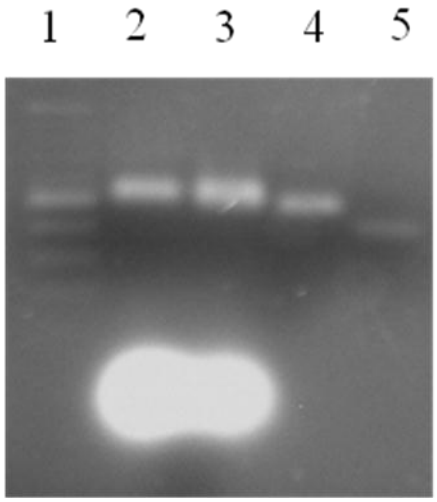
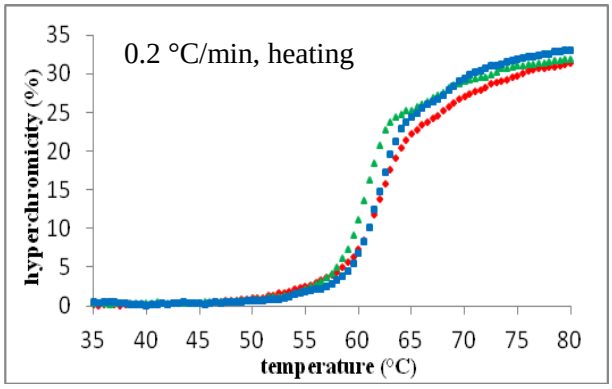


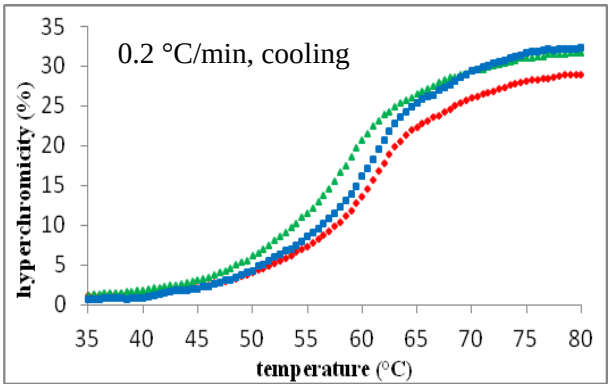
Fig. S5. Fluorescence image of an agarose gel with ethidium bromide staining. *Lane 1*: 100 bp ladder; *Lane 2*: 0.5 pmol linear M1.3, assembled with 50 equivalents of staple strands with **PyC** 5'-caps; *Lane 3*: 0.5 pmol linear M1.3, assembled with 50 equivalents staple strands with **PyPy** 5'-caps; *Lane 4*: 0.5 pmol linear M1.3, assembled with 10 equivalents of *unmodified* staple strands; *Lane 5*: 0.5 pmol linear M1.3 scaffold alone.

8. Melting curves of 4F M1.3 Origami with and without 5'-capped staple strands

a)



b)



c)

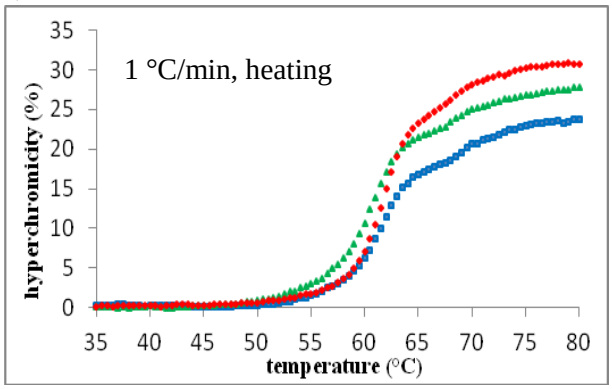


Fig. S6. UV-melting curves for unmodified 4F M1.3 origami (*green*), 4F M1.3 with globally 5'-**PyPy** capped staples (*blue*), and 4F M1.3 with globally 5'-PyC capped staples (*red*), measured at 260 nm at 50 nM concentration of M1.3 in each experiment.. a) Heating curves at a rate of 0.2 °C/min, (b) cooling curves at a rate of 0.2 °C/min, (c) heating curves at a rate of 1 °C/min.

The curves shown in a), b), and c) are the primary data for the first derivatives shown in Fig. 4 of the main paper. For an additional set of curves and their first derivative (fast cooling), see Figure S7, below.

Additional melting curves

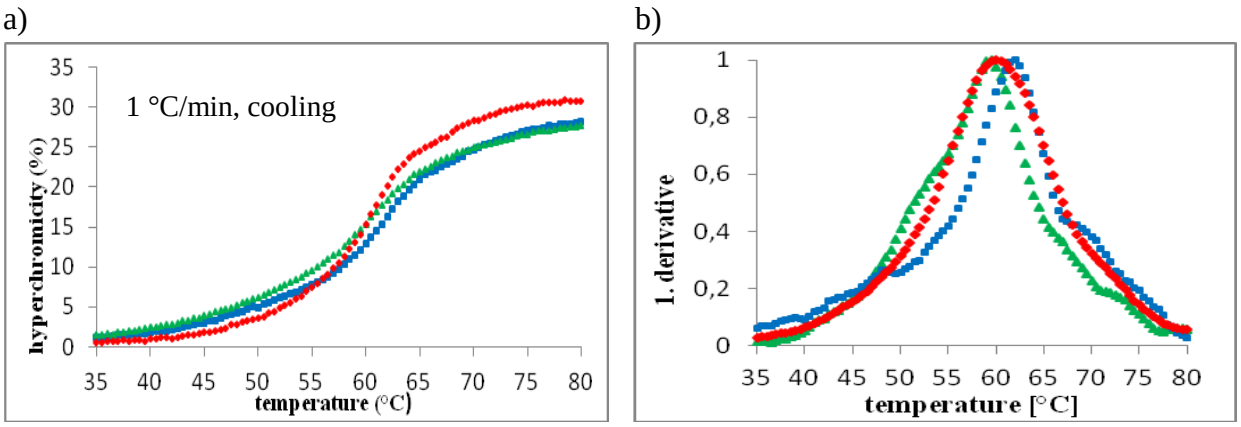


Fig. S7. UV-melting curves for unmodified 4F M1.3 origami (*green*), 4F M1.3 with globally 5'-**PyPy** capped staples (*blue*), and 4F M1.3 with globally 5'-PyC capped staples (*red*), measured at 260 nm at 50 nM concentration of M1.3 in each experiment.
(a) Cooling curves at 1 °C/min. (b) First derivative of the melting curve shown in a).
This is the set of cooling curves that corresponds to the melting curves shown in Fig. 4b (main paper) and Fig. S6c (above).

9. UV-Melting points and melting transition breadths of 4F sheets

Table S1. UV melting points (T_m) of unmodified 4F M1.3 origami (4F) and 4F M1.3 origami with globally 5'-**PyPy** capped staples (4F PyC) or globally 5'-**PyC** capped staples (4F PyPy) at a heating and cooling rate of 0.2 °C/min or 1 °C/min. Data are the average of two measurements.

Sample	T_m melting (°C) at 1 °C/min	T_m melting (°C) at 0.2 °C/min	T_m cooling (°C) at 1 °C/min	T_m cooling (°C) at 0.2 °C/min
4F	60	60	59.5	58
4F PyC	62	62	61	61
4F PyPy	61.5	62	62	61

Table S2. Cooperativity of melting transition, as determined by the breadth of UV-melting transitions ("transition breadths", Tb) of unmodified 4F M1.3 origami (4F) and 4F M1.3 origami with globally 5'-**PyPy** capped staples (4F PyC) or globally 5'-**PyC** capped staples (4F PyPy). Data are based on the curves shown in Fig. 4 of the main paper and Fig. S7b, above). For an early example of analyzing the melting transition breadths of DNA duplex-mediated nanostructures, see ref. 6.

Sample	T_b melting (°C) at 1 °C/min	T_b melting (°C) at 0.2 °C/min	T_b cooling (°C) at 1 °C/min	T_b cooling (°C) at 0.2 °C/min
4F	5.9	5.5	12.7	11.2
4F PyC	11.2	6.5	13.4	9.5
4F PyPy	7.7	6.1	9.8	10.1

10. MALDI spectra of individual 5'-capped oligonucleotides

Shown below are mass spectra of oligonucleotide sequences that were 5'-capped in parallel to the global capping of mixtures of support-bound staple strands. The latter are very difficult to analyze by MALDI-TOF mass spectrometry due to the large number of signals and the large differences in desorption/ionization yield for the different sequences involved. The MALDI-mass spectra were recorded on a Bruker REFLEX IV spectrometer in linear negative mode using 2,4,6-trihydroxyacetophenon monohydrate (THAP, 0.3 M in ethanol) and diammonium citrate (0.1 M in water) in ratio (2:1 v/v) as matrix/comatrix, with an accuracy of ±0.1% of the mass.

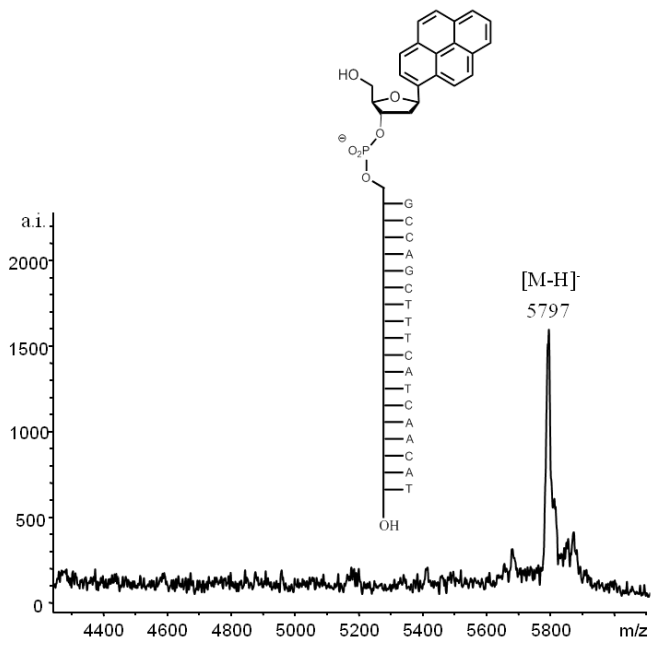


Fig. S8. MALDI-TOF mass spectrum of 5'-PyC-GCCAGCTTTCATCAACAT-3'. For the pseudomolecular ion $[M-H]^-$: calcd 5798, found 5797

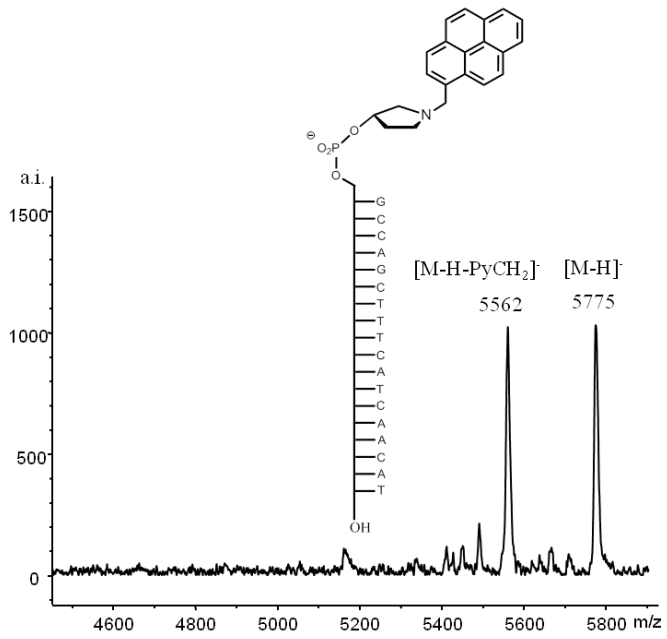


Fig. S9. MALDI-TOF mass spectrum of 5'-PyPy-GCCAGCTTTCATCAACAT-3'. Note that the pyrenyl cap fragments, as described in the literature.[2] For the unfragmented pseudomolecular ion $[M-H]^-$: calcd 5778.7, found 5775.

11. Reference for Supporting Information

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