

Supporting Information

**Programmed Dissociation of Dimer and Trimer
Origami Structures by Aptamer-Ligand Complexes**

*Na Wu and Itamar Willner**

Institute of Chemistry, The Center of Nanoscience and Nanotechnology,

The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Address correspondence to: willnea@vms.huji.ac.il

Tel: +972-2-6585272

Fax: +972-2-6527715

Description		Origamis Counted	
T ₁ -T ₂	Prior to cleavage	Singles	30
		Dimers	248 (89%)
		In all	278
	After interaction with ATP	Singles	186
		Dimers	58 (24%)
		In all	244

Figure S1. The detailed analysis of large-area domains: the ATP-programmed cleavage of the dimer origami structure T₁-T₂.

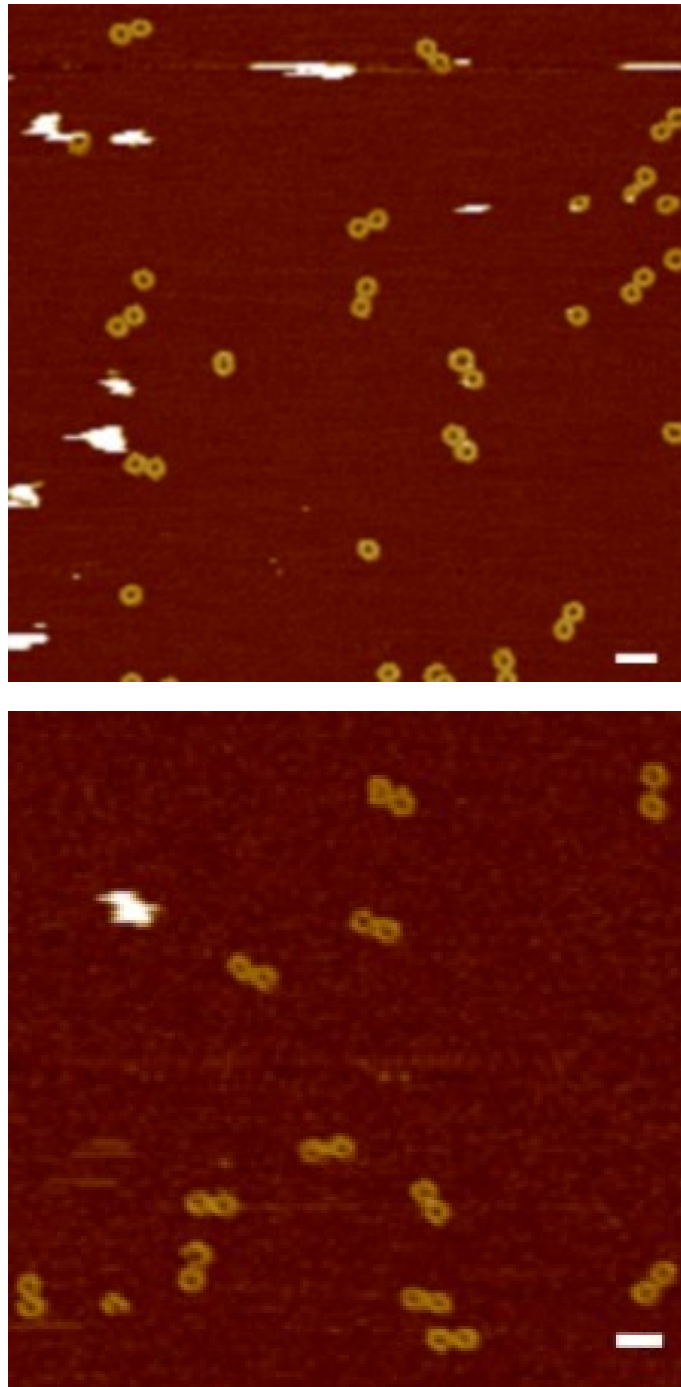


Figure S2. AFM images corresponding to the diluted sample depicted in Figure 1 (B). (3 - fold dilution). The images show that no apparent tetramers are observed, as discussed in the text. Scale bars: 200 nm.

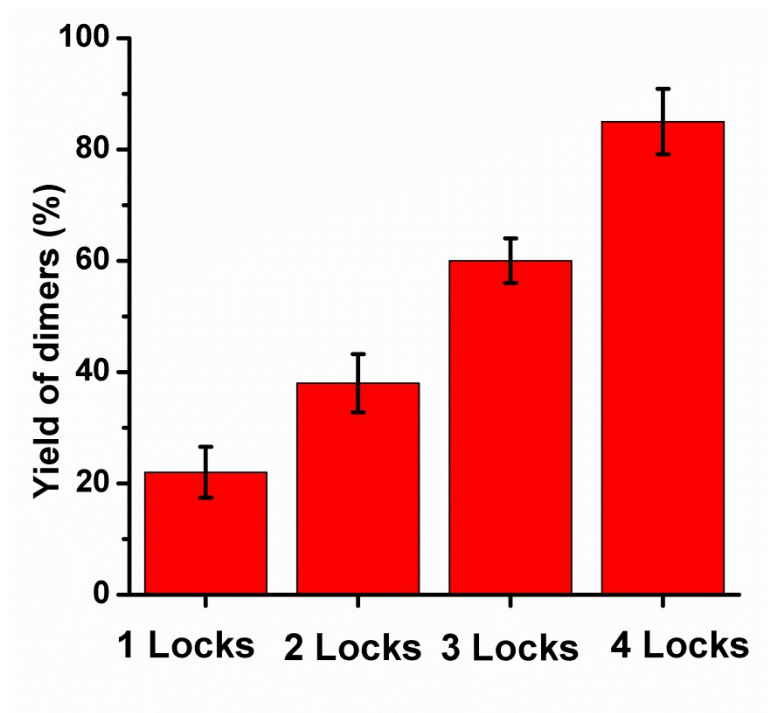


Figure S3. The yields of the origami dimers (T_1 - T_2) as a function of the number of duplex crosslinking units (each duplex includes 11 base-pairs).

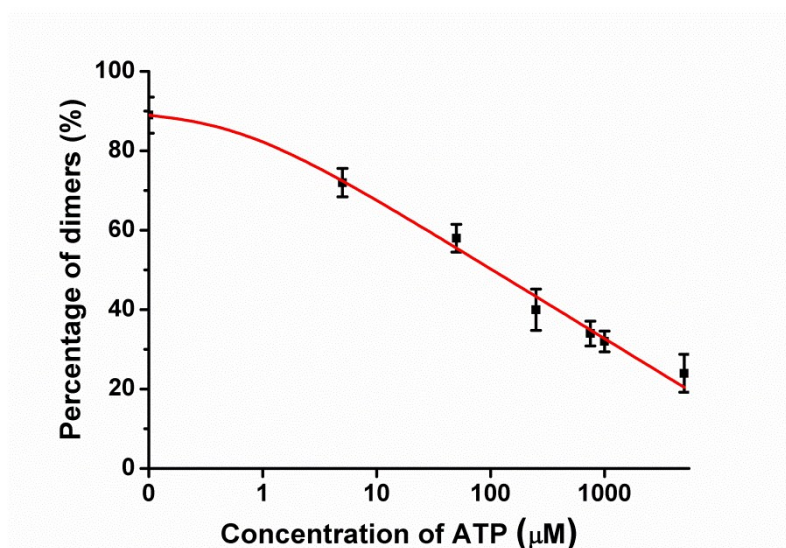


Figure S4. Percentage of AFM-imaged T_1 - T_2 dimer structures upon subjecting the T_1 - T_2 origami mixture to variable concentrations of ATP, for a fixed time-interval of two hours.

Description	The number of Origami tiles / Proportion		
	Singles	Dimers	In all
Prior to cleavage	30	248/89%	278
After interaction with 5 μ M ATP	92	242/72%	334
After interaction with 50 μ M ATP	279	392/58%	671
After interaction with 250 μ M ATP	200	134/40%	334
After interaction with 750 μ M ATP	338	172/34%	510
After interaction with 1 mM ATP	189	88/32%	277
After interaction with 5 mM ATP	186	58/24%	244

Table S1. The detailed analysis of large-area domains: the concentration-dependent (ATP) cleavage of the dimer origami structure T_1 - T_2 .

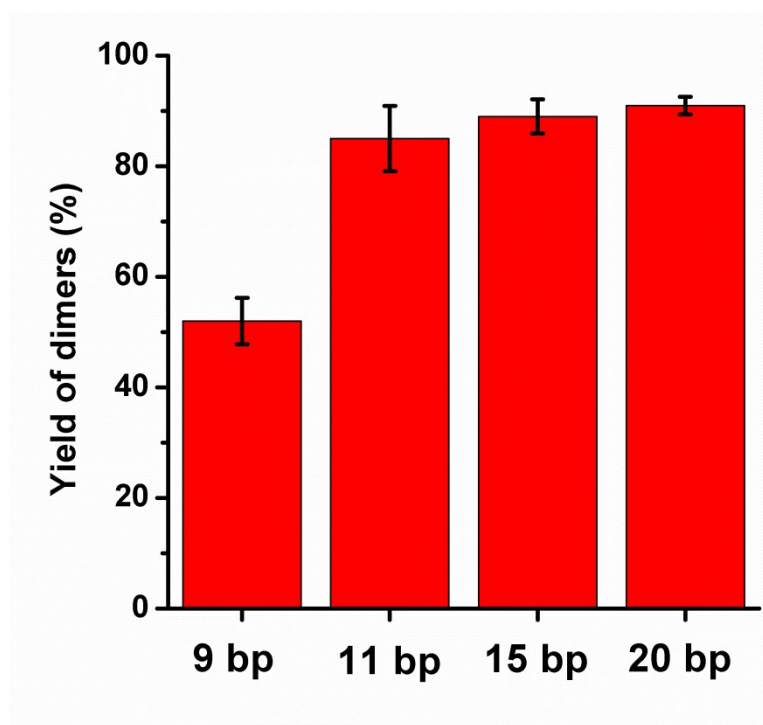


Figure S5. The yields of the origami dimers (T_1 - T_2) as a function of the number of base pairs, upon applying four duplex crosslinking bridges.

Description		Origamis Counted	
T ₃ -T ₄	Prior to cleavage	Singles	53
		Dimers	340 (87%)
		In all	393
	After interaction with Cocaine	Singles	168
		Dimers	84 (33%)
		In all	252

Figure S6. The detailed analysis of large-area domains: the cocaine-programmed cleavage of the dimer origami structure T₃-T₄.

Description	The number of Origami tiles / Proportion		
	Singles	Dimers	In all
Prior to cleavage	53	340/87%	393
After interaction with 5 μ M cocaine	117	238/67%	355
After interaction with 50 μ M cocaine	91	134/60%	225
After interaction with 250 μ M cocaine	83	94/53%	177
After interaction with 750 μ M cocaine	122	104/46%	226
After interaction with 1 mM cocaine	122	92/43%	214
After interaction with 5mM cocaine	168	84/33%	252

Table S2. The detailed analysis of large-area domains: the concentration-dependent (cocaine) cleavage of the dimer origami structure T₃-T₄.

Description	The number of Origami tiles / Proportion						
	$T_1-T_5-T_4$	T_1-T_5	T_5-T_4	T_1	T_5	T_4	In all
Prior to cleavage	216/80%	16/5.9%	8/3.0%	10/3.7%	7/2.6%	12/4.5%	269
After interaction with ATP	66/19%	28/7.9%	142/40%	66/19%	28/7.9%	24/6.8%	354
After interaction with cocaine	60/19%	120/39%	20/6.5%	23/7.4%	22/7.1%	65/21%	310
After interaction with ATP & cocaine	6/2.8%	24/11%	28/13%	44/20%	53/25%	60/28%	215

Figure S7. The detailed analysis of large-area domains: the ATP or/and cocaine-programmed cleavage of the trimer origami structure $T_1-T_5-T_4$.

Experimental Section

The DNA origami tiles were assembled in a TAE buffer solution consisting of Tris buffer 20 mM that included acetic acid 20 mM, EDTA 1 mM, magnesium acetate, 12.5 mM, pH = 8.0. Single-stranded M13mp18 phage DNA, 5 nM, (New England Biolabs), was dissolved in the buffer solution, and the short staple strands (unmodified staple strands, functionalized specific edge staple strands and biotinylated staple strands) (Integrated DNA Technologies), 50 nM were added to the M13mp18 buffer solution. The mixture was heated to 95 °C in a thermal cycler and then allowed to cool down to 20 °C at a rate of 0.1 °C/10 seconds. The respective origami-tile samples were purified using 100 kD MWCO centrifuge filters to eliminate the excess of unreacted staple strands.

The respective origami monomers were mixed at the same concentration ratio in 1×TAE/Mg²⁺ buffer to form the corresponding dimer or trimer structures, after heating the mixture to 45 °C, and allowing it cooling to 15 °C at a rate of 0.1 °C/minute for the assembly of the dimers, and at a rate of 0.05 °C/minute for the assembly of the origami trimers. The trimer origami structure was purified by electrophoresis and

extracting from the gel using Freeze 'N Squeeze spin columns (BioRad). The separated trimer cut out of the gel is depicted in Figure 6, lane 5.

For the cleavage of the origami dimers or trimers in HEPES/Mg²⁺ buffer solution that included HEPES 20 mM, MgCl₂ 12.5 mM, pH = 7.0. ATP or/and cocaine was added to the origami TAE/Mg²⁺ solution, the volume of the origami solution was five – fold diluted by the added HEPES/Mg²⁺ buffer solution. The cleavage was allowed to proceed for 2 hours, at 30 °C. The dissociation yield as a function of ATP/cocaine concentration was probed in HEPES/Mg²⁺ buffer solution, for a time-interval of 2 hours, at 30 °C.

The AFM imaging of the different origami samples was performed using a Multi-mode Nanoscope VIII AFM (Bruker) and DNP-S probes (Bruker). Before imaging, streptavidin solution was added into the origami samples, incubating for 5 minutes. Then, a drop, 2 µl, of the respective origami-tiles was deposited on freshly-cleaned mica, and allowed to adsorb for 5 minutes. Imaging was performed by covering the surface with the TAE/Mg²⁺ buffer solution.

Electrophoretic experiments were performed using 1% agarose gel, subjected to a constant voltage of 80 V at 0 °C for 1.5 h, in 1×TAE/Mg²⁺ buffer. The gel was stained by SYBR gold to identify the separated bands.

DNA sequences:

Staple Strands for Hexagonal Origami Structure and Modified Staple Strands

A01	ACAGGTCAGAACCAGACCGGAAGCCCGCTTTT
A02	TAGTCAGAATCAGGTCTTTACCCTAACGGGT
A03	TAGACTGGCAGAGGGGTAATAGTATTTGTAT
A04	CAGATACATAGGAATACCACATTCGCATAGG

A05 TTCAAAGCGGATTAGAGAGTACCTTTAATTGC
A06 AATCAAAAAGCAAAGCGGATTGCAATTCGAGC
A07 AGTTTTGCATAGCGTCCAATACTGTGACCATA
A08 TTGAGATTTAACGCCAAAAGGAATGCAAAAGA
A09 CATTGTGAATTACCTTATGCGATTTTCATCAG
A10 ACTTCAAATATCGCGTTTTATCAAAAAG
A11 ATTAAGAGGTTCCAGAAAACGAGAACGGAATCG
A12 TCATAAATATAGCGAGAGGCTTTTTACGAGGC
A13 ATAGTAAGATTACAGGTAGAAAGATTAAGAAC
A14 ACAACATTAGCAACACTATCATAAGACGATAA
A15 TGGCTCATTATACCAGTCAGGACGCTAACGGA
A16 GCGGGATCTGCAGGGAGTTAAAGGAAACTCCA
A17 AAAATACGTGAGGAAGTTTCCATTGACTATTA
A18 CATCGCCTACAAAGTACAACGGAGAAAATGTT
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A20 TGGGCTTGAGATGGTTTAATTTCACTTTAAT
A21 CTGAGGCTGTCACCCTCAGCAGCGACTAAAGA
A22 CTTTTTCATAATGCCACTACGAAGTTATACCA
A23 AGCGCGAAGATAAATTGTGTGCGAAAAAGAGGA
A24 CAGATGAACCTTCATCAAGAGTAATAGTAAAT
A25 CTA AACGTACAGAGGCTTTGAGGAAAGACAGCATCGGAACGAG
A26 CCTGCTCCCTTTGACCCCCAGCGAGCACCAAC
A27 AGAACCGGGAAGTACCAACTTTGATCCGCGA
A28 CCTGACGAGAAACACCAGAACGAGTCTTGACA
A29 ACACTCATATGTTACTTAGCCGACAATCATA
A30 AGGGAACCATATTCATTACCCAAAAGGCTTGC
B01 ATACCGATGAGGTGAATTTCTTAACCAGTAGC
B02 GCGAATAATAGAAAGGAACAAGTACGCGTGT
B03 TAGCGTAACATTCCACAGACAGCCCCACCCTC
B04 CACCCTCATAGTACCGCCACCCTCGCGCAGTC
B05 TTGCTTTAGTTGCGCCGACAATGACAACAAC
B06 AGTGAGAATAATTTTTTACGTTGTTATCAGC
B07 GCCTGTAGCGATCTAAAGTTTTGTTTCAGCGG
B08 AGGAGGTTGAACCGCCACCCTCAGACTACAAC
B09 GTATTAAGAGGCTGAGACTCCTCACCGTACTC
B10 GAGCCTTTAATTGTATCGGTAAAATCTC
B11 CAAAAAAAACAACCTTTCAACAGTCGTCTTTC
B12 CAGACGTTTTTCGTCACCAGTACAAAGCCACCA
B13 CCCTCATTCGGAATAGGTGTATCAAGAGAAGG
B14 GTATAGCCTTCAGGGATAGCAAGCTACCGTAA
B15 ATTAGGATTAGCGGGTTTTGCTCTGATATAA
B16 ACCATTACAGAGCCAGCAAAATCAACAGCTTG
B17 TCATCGGCTTAGCGTCAGACTGTAAAGGAATT
B18 AGAGCCACGCCACCCTCAGAACCGCTCATAGT

B19 TCTGAATTAAGCCAGAATGGAAAAGAACCGC
 B20 TATTCGGAACCTATTATTCTGAAACATGAAA
 B21 TGGGAATTCATTAGCAAGGCCGGAAGAATCAA
 B22 GTTTGCCTATTTTCGGTCATAGCCCGCCTCCC
 B23 TCAGAGCCACCCCTCAGAGCCGCCAAATAAA
 B24 TCCTCATTTACCGTTCCAGTAAGCCCCCTGCC
 B25 AGCGTTTGCCTAATCAGTAGCGACAACGTCACCAATGAAACCAT
 B26 CACCACCAGAGCCACCACCGGAACCCCTTATT
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 B28 AGTGCCCGTATAAACAGTTAATGCGTCATACA
 B29 CGGAACCAGAGCCGCCGCCAGCATCAGGTCAG
 B30 ACGATTGGGATGATACAGGAGTGTGAGTAAC
 C01 AGGTAATACATTCAACCGATTGACAACAGTA
 C02 GAAACGCAACATAAAGGTGGCAACAGTAATTC
 C03 CCAGAAGGTAAGCAGATAGCCGAAACCAATCA
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linker AB1 CATCGCCCACGCATAACCGATATATTCGGTTCG
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C03-Biotin	Biotin-TTTTTCCAGAAGGTAAGCAGATAGCCGAAACCAATCA
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L ₁ 'A15-9bp	GGTGGACAATTTTTGGCTCATTATACCAGTCAGGACGCTAACGGA
L ₁ 'A20-9bp	GGTGGACAATTTTTGGGCTTGAGATGGTTAATTTCAACTTTAAT
L ₁ 'A28-9bp	GGTGGACAATTTTTCTGACGAGAAACACCAGAACGAGTCTTGACA
L ₁ 'A-8lock-1	CAGGTGGACAATTTTTATGCGATTTTCATCAG
L ₁ 'A-8lock-2	CAGGTGGACAATTTTTTCAGGACGCTAACGGA
L ₁ 'A-8lock-3	CAGGTGGACAATTTTTAATTTCAACTTTAAT
L ₁ 'A-8lock-4	CAGGTGGACAATTTTTAGAACGAGTCTTGACA
L ₁ 'A-8lock-5	CAGGTGGACAATTTTTTCATTGTGAATTACCTT
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L ₁ 'A-8lock-8	CAGGTGGACAATTTTTCTGACGAGAAACACC
L ₁ D-8lock-1	TTGTCCACCTGGGGGAGTATTGCGGAGGAAGGTGTTTTTACCTTTTAAAGAAGA
L ₁ D-8lock-2	TTGTCCACCTGGGGGAGTATTGCGGAGGAAGGTGTTTTTCGCTGAGAGGCGAA

L ₁ D-8lock-3	TTGTCCACCTGGGGGAGTATTGCGGAGGAAGGTGTTTTTAGGTTAACGTCAGAT
L ₁ D-8lock-4	TTGTCCACCTGGGGGAGTATTGCGGAGGAAGGTGTTTTAAAACAGACAATTCAT
L ₁ D-8lock-5	TTGTCCACCTGGGGGAGTATTGCGGAGGAAGGTGTTTTGAATATACAGTAACAG
L ₁ D-8lock-6	TTGTCCACCTGGGGGAGTATTGCGGAGGAAGGTGTTTTAGAAACAATAACGGAT
L ₁ D-8lock-7	TTGTCCACCTGGGGGAGTATTGCGGAGGAAGGTGTTTTAATTGCGTAGATTTTC
L ₁ D-8lock-8	TTGTCCACCTGGGGGAGTATTGCGGAGGAAGGTGTTTTAAAATTATTTGCACGT