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Supporting Information

Disassembly of DNA Origami Dimers Controlled by Programmable Polymerase Primers

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Content

1. Materials and Methods

The formation of the cross-shaped DNA origami unit. All of the origamis used in this paper were designed with the software caDNAno (http://cadnano.org/). All DNA strands were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The connecting strands were ordered with ULTRAPAGE-purification, the staple strands and the primer strands were ordered with HAP-purification, and modified strands (with FAM or BHQ1) were ordered with HPLC-purification. The cross-shaped DNA origami was assembled by a slow cooling method. To construct the cross-shaped origami-A, 20nM M13mp18 strands were mixed with 197 staple strands and 4 connecting strands at a molar ratio of 1:5:5, in 1×TAE buffer solution containing 40 mM Tris-HCl, 20 mM of acetic acid, 2 mM of EDTA, and 12.5 mM of magnesium acetate. To construct the cross-shaped origami-B, 20nM M13mp18 strands were mixed with 197 staple strands at a molar ratio of 1:5, in the same 1×TAE buffer solution. The mixtures were annealed from 90 °C to 24 °C within ~10 h (Table S3). The DNA origami was then purified to remove excess staple strands, using 100kDa ultracentrifugal filters (MWCO, Amicon, Millipore, Molsheim, France).

The assembly of the DNA origami dimer. The DNA origami concentration was determined by the estimated extinction coefficient at 260 nm (~1.091 × 108 $M^{-1}cm^{-1}$). The concentration of the origami

structure was ~30 nM. The prepared DNA origami monomers was mixed at a molar ratio of 1:1 in 1×TAE buffer solution containing 10 mM of magnesium acetate. Then, the mixture was annealed from 45℃ to 25℃ at a rate of 0.05℃/min to form the corresponding DNA origami dimers. The assembled DNA origami dimers were directly used for AFM images, without further purification.

The disassembly of the DNA origami dimer. The concentration of the DNA origami dimers was estimated to be ~15 nM. The DNA origami dimers and primer strands were mixed together at a molar ratio of 1:4.8. Then, the mixture was annealed from 38℃ to 25℃ at a rate of 0.1℃/min to bind the primer strands with the connecting strands. The samples were mixed with 8 kU/L, 16 kU/L, 32 kU/L, or 64kU/L of Klenow polymerase (Thermo Fisher Scientific, Shanghai, China) in 1 × Klenow buffer. The dNTP concentration was 5 times (including all four kinds of deoxyribonucleotides) that of the origami dimers, approximately 75 nM. Mixed samples were incubated for 6 hours at 37 °C. The disassembled DNA origami dimers were directly used for AFM images, without further purification.

AFM imaging. Samples (5 μL) were dripped onto freshly cleaved mica and kept for 5 min. Then, 20 μL 1 × TAE (with 10 mM magnesium acetate)

was added to the scanned area. All images were obtained by a Bruker Multimode 8 AFM (Bruker Corporation, Germany), and scanned in "ScanAsyst in fluid" Mode.

Fluorescent experiments. All experiments were performed at 37℃ in 1× Klenow buffer using a real-time fluorescence PCR (ABI, Step-one Plus). In a typical reaction, the total volume of the solution was 30 μL for detection. The samples with primer "ab", "ba", "aa", "bb", and "abc" were mixed with 8 kU/L of Klenow polymerase in 1 × Klenow buffer. The sample with primer "cab" was mixed with 64 kU/L of Klenow polymerase in 1 × Klenow buffer (for the explanation for choosing the concentration of Klenow, see Figure S17). The fluorescence intensity was measured every 4 minutes for ~8 hours. Each experiment was repeated twice to ensure reproducibility. In each reaction, the fluorescence intensity was calculated by subtracting the fluorescence intensity baseline at each time point and was normalized. The error bars stand for the standard errors.

2. Supporting Figures

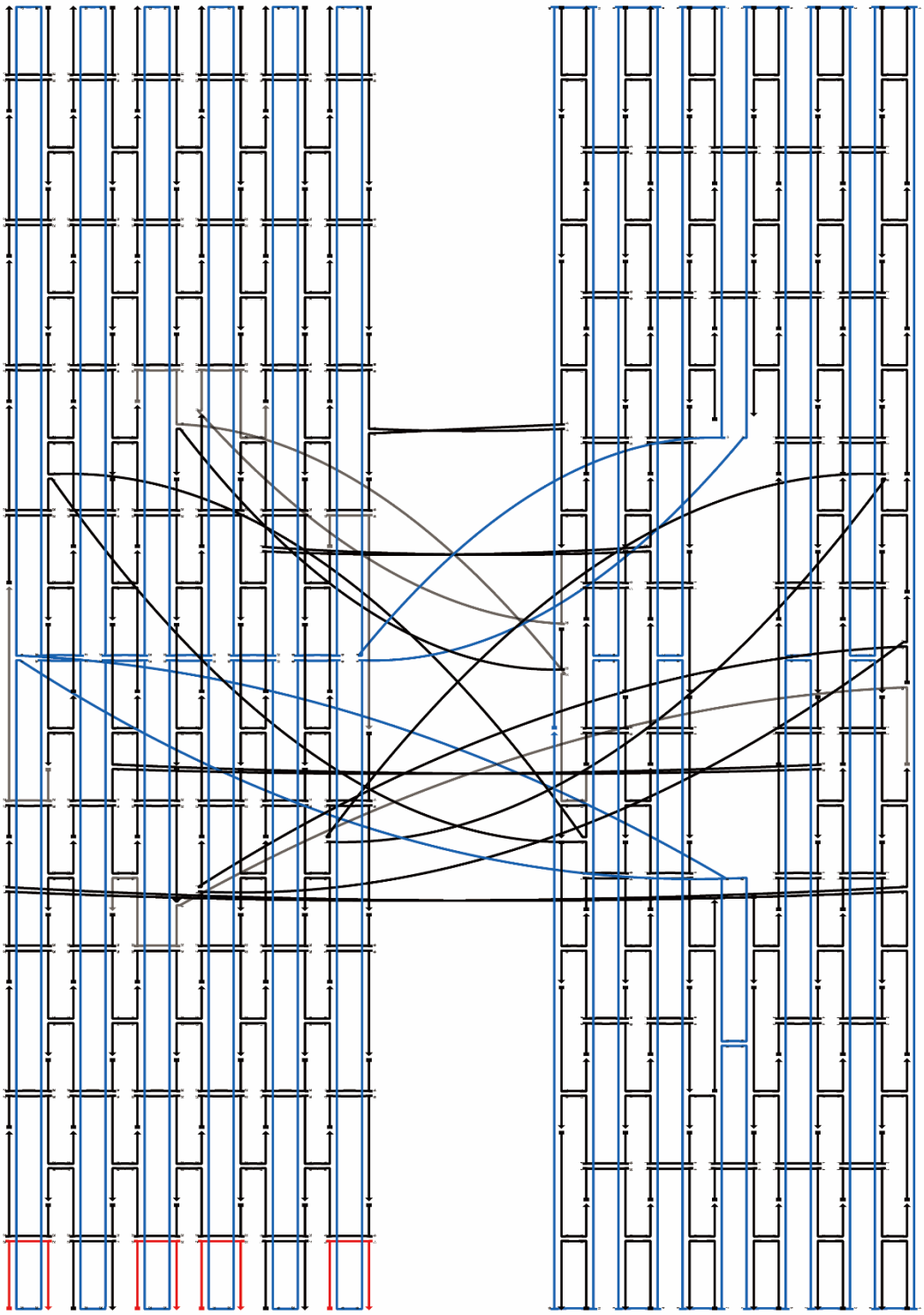

Figure S1. Layout of the cross-shaped origami-A. Blue line: scaffold (M13mp18); black line: the staple strands; red line: the domain x of the connecting strands.

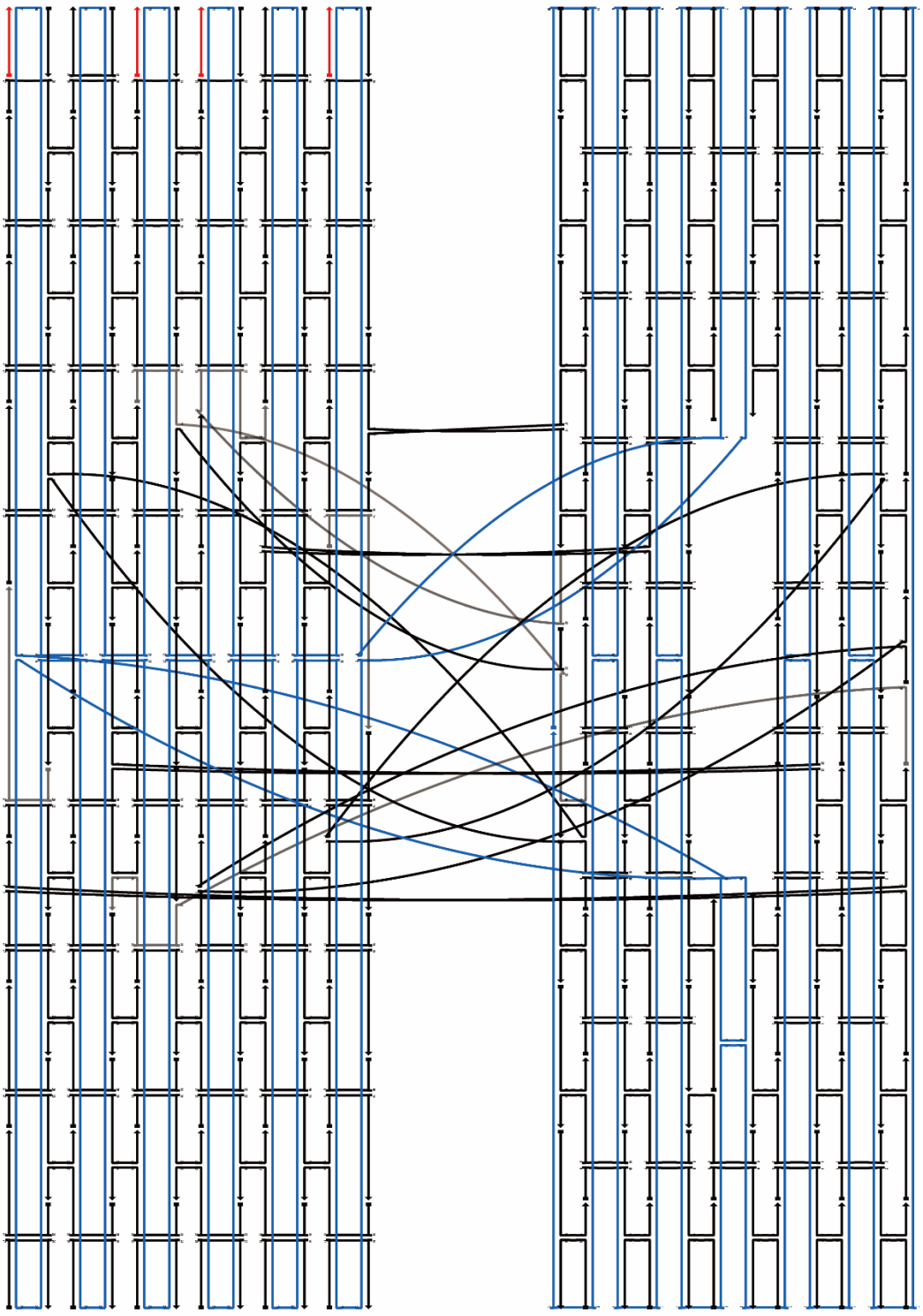

Figure S2. Layout of the cross-shaped origami-B. Blue line: scaffold (M13mp18); black line: the staple strands; red line: the domain y of the connecting strands.

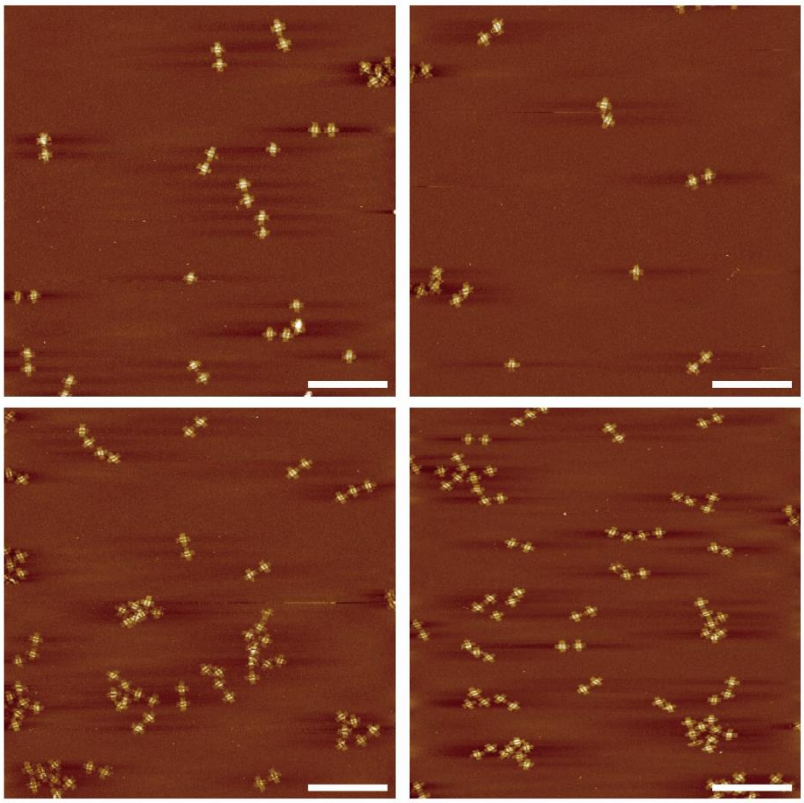

Figure S3. AFM images of the origami dimers before the polymerase-triggered strand displacement disassembly. Scale bars, 400 nm.

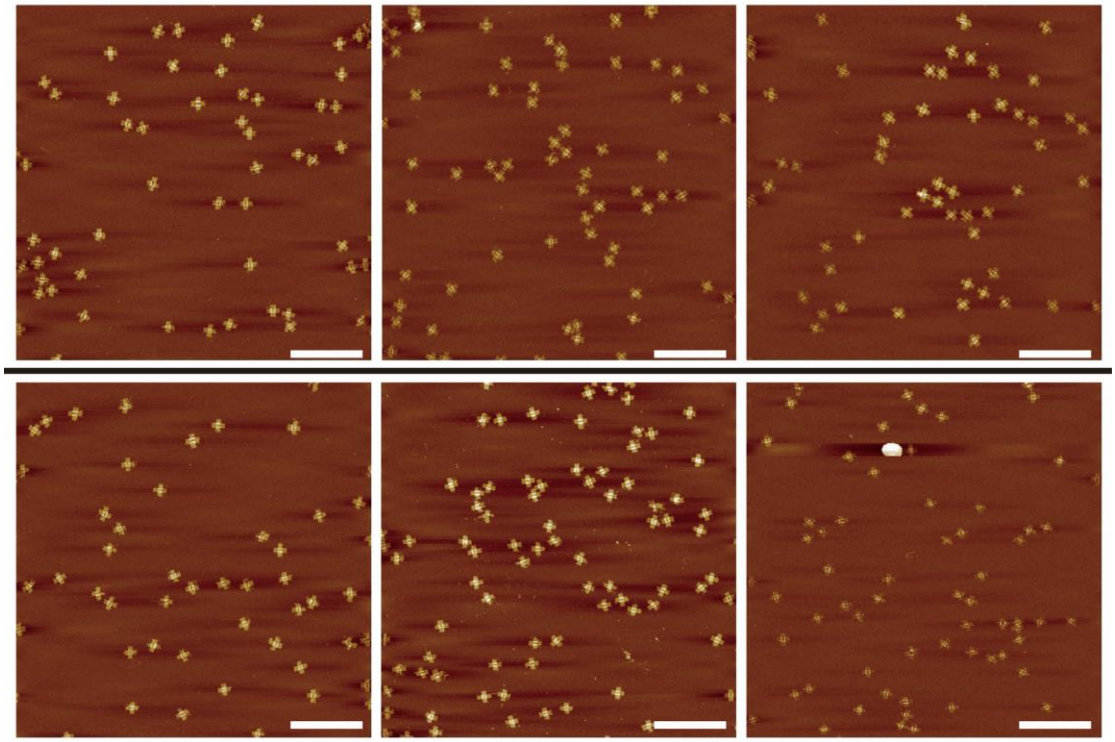

Figure S4. AFM images of the monomer frames after the origami dimers were treated with the primer strand and Klenow polymerase, 16 kU/L (Up) and 32 kU/L (Down), respectively, incubating for 6 h at 37℃. Scale bars, 400 nm.

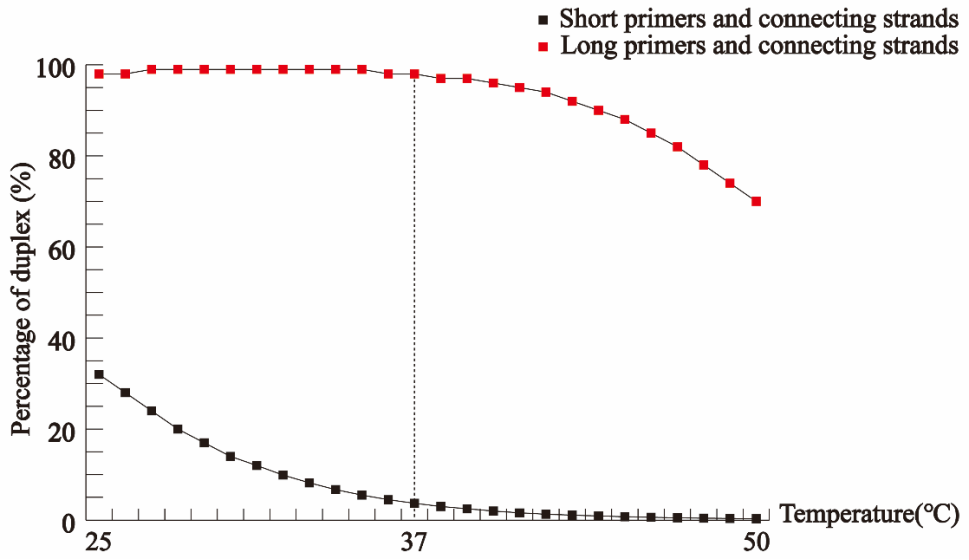

Figure S5. Percentage of duplexes of primer and connecting strands upon subjecting short or long primer strand and connecting strand to variable temperatures simulated by NUPACK (http://nupack.org/). The primer and connecting strands were at a molar ratio of 1.2:1.

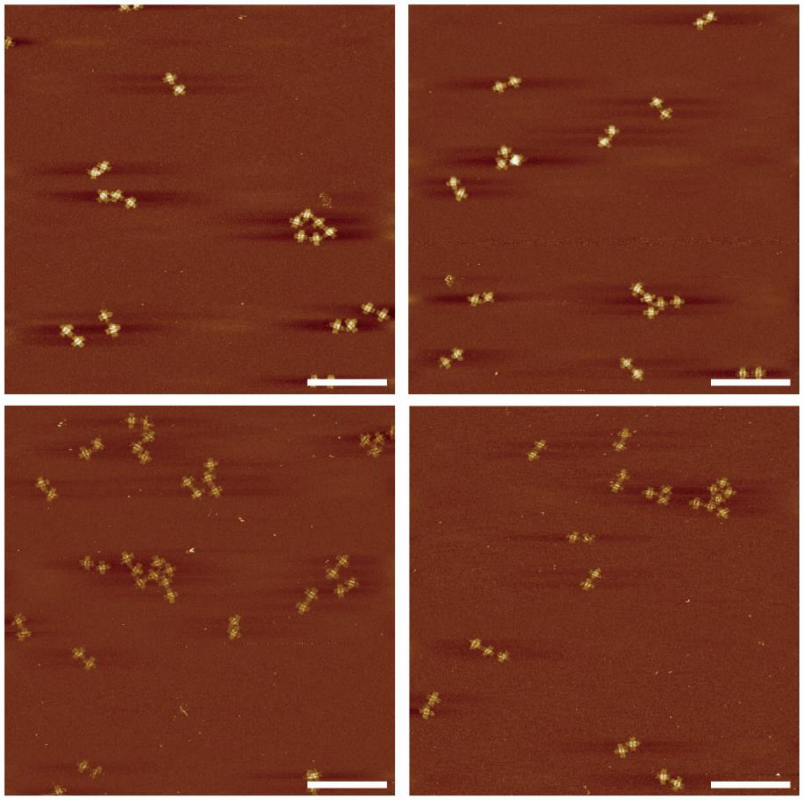

Figure S6. AFM images of the origami dimers before the disassembly of the origami dimers with programmable primers and Klenow polymerase. Scale bars, 400 nm.

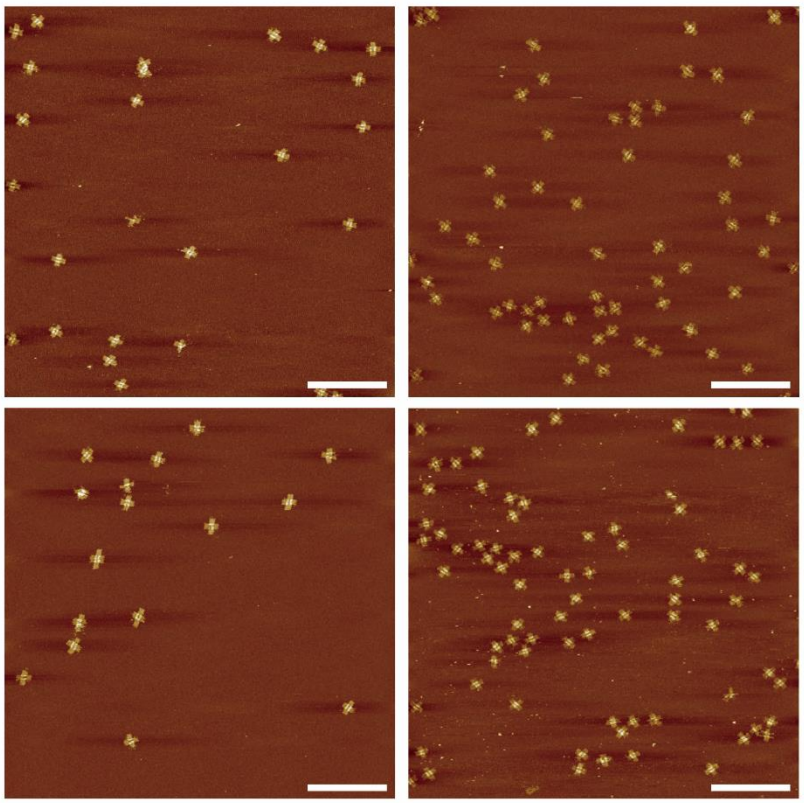

Figure S7. AFM images of the origami dimers after treatment with the primer strand “ab” and Klenow polymerase, 32 kU/L, incubating for 6 h at 37℃. Scale bars, 400 nm.

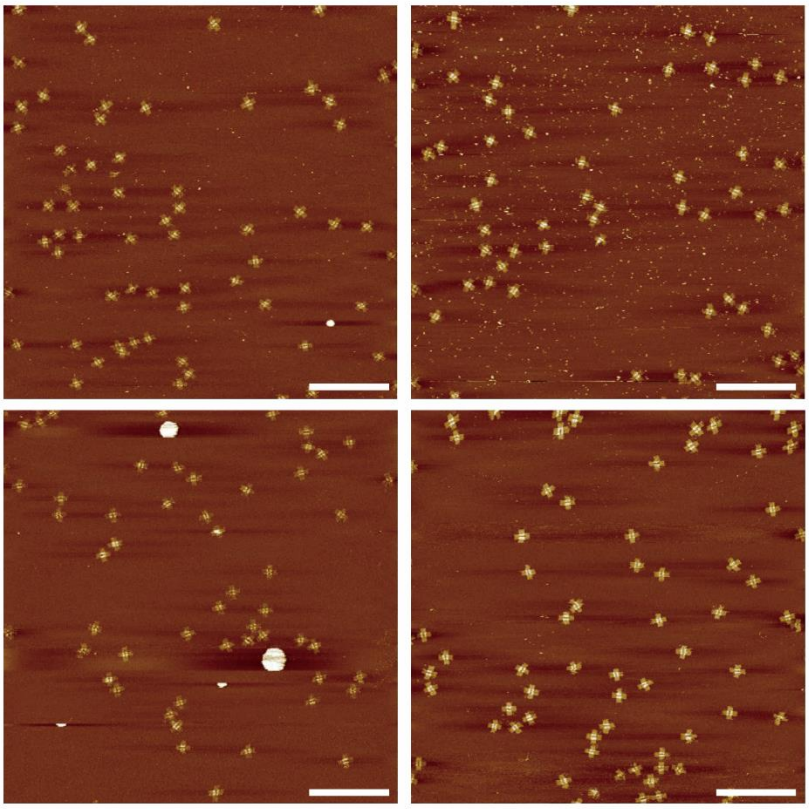

Figure S8. AFM images of the origami dimers after treatment with the primer strand “ba” and Klenow polymerase, 32 kU/L, incubating for 6 h at 37℃. Scale bars, 400 nm.

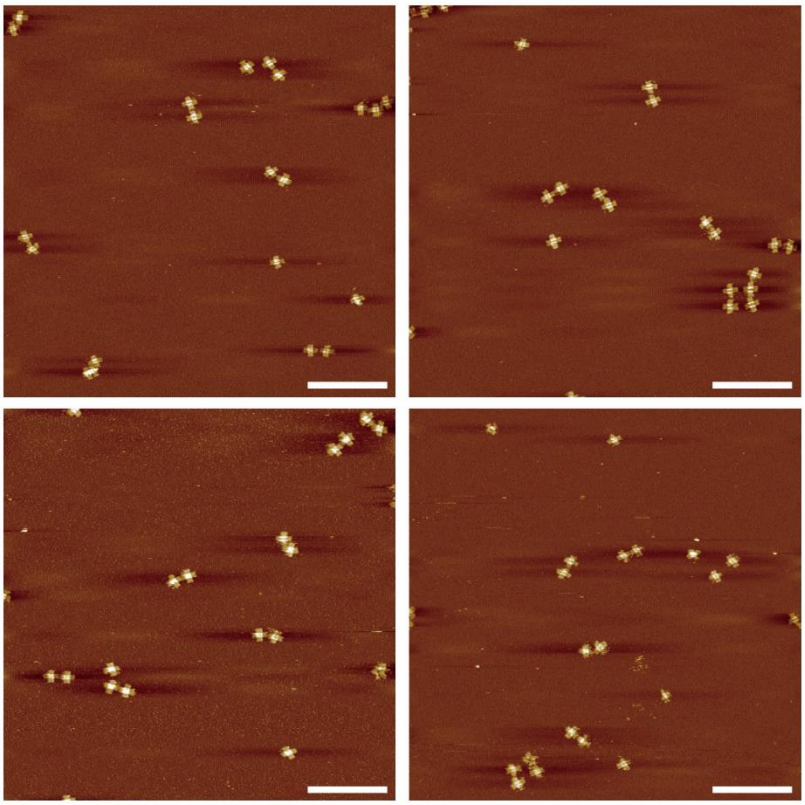

Figure S9. AFM image of the origami dimers after treatment with the primer strand “aa” and Klenow polymerase, 32 kU/L, incubating for 6 h at 37℃. Scale bars, 400 nm.

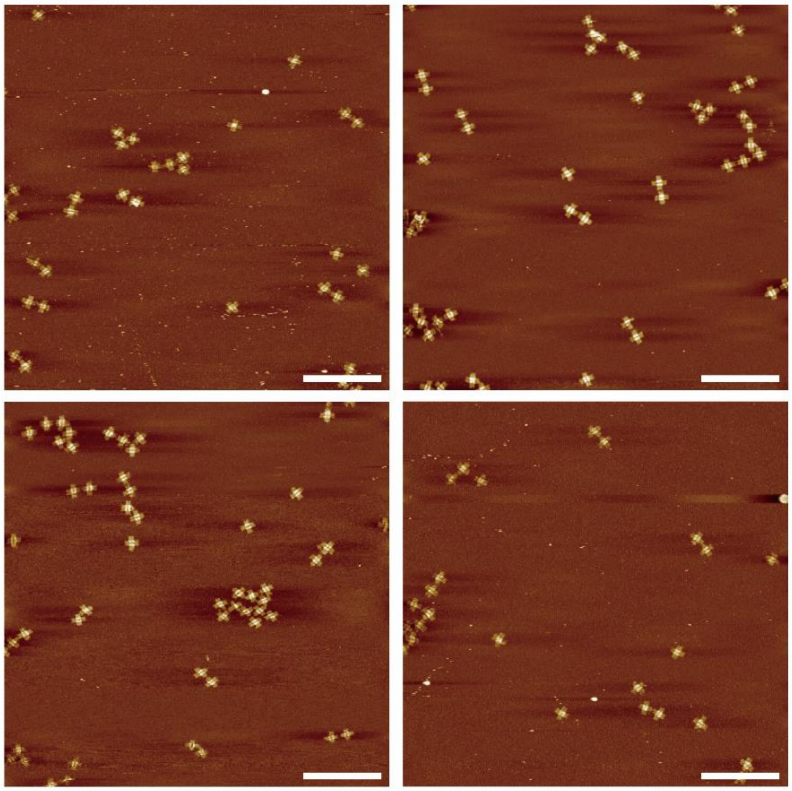

Figure S10. AFM image of the origami dimers after treatment with the primer strand “bb” and Klenow polymerase, 32 kU/L, incubating for 6 h at 37°C. Scale bars, 400 nm.

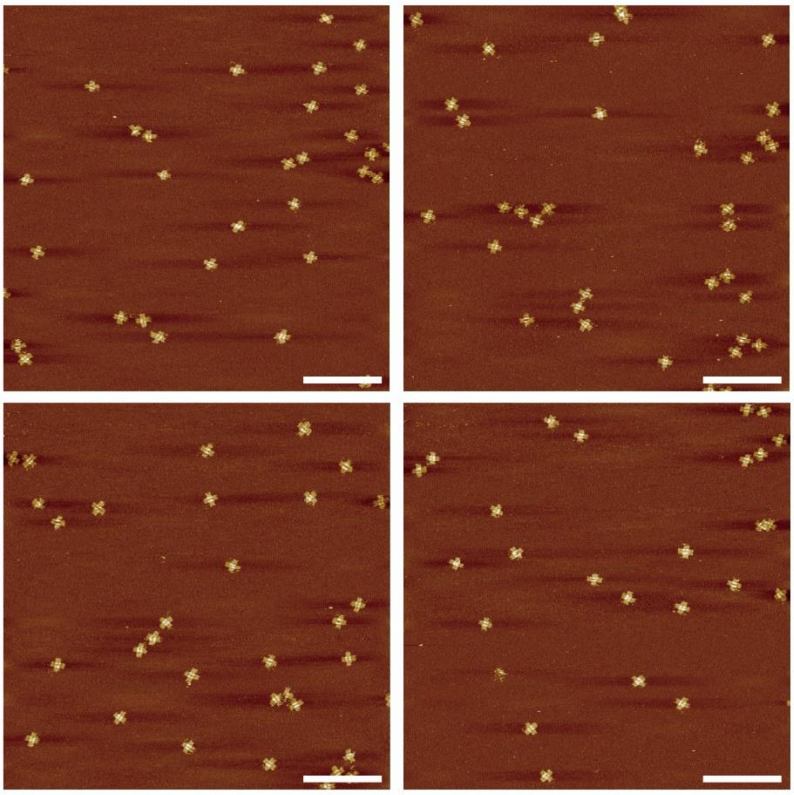

Figure S11. AFM image of the origami dimers after treatment with the primer strand “abc” and Klenow polymerase, 32 kU/L, incubating for 6 h at 37°C. Scale bars, 400 nm.

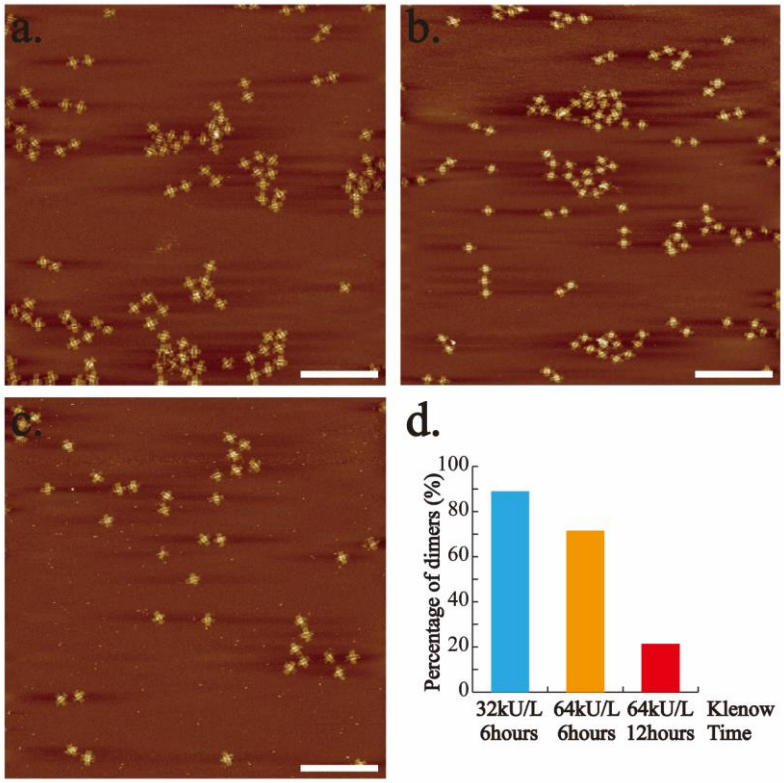

Figure S12. (a-c) AFM images of the DNA origami dimers after polymerization with the primer strand "cab" and Klenow polymerase at 37℃. 6h polymerization and 32 kU/L Klenow polymerase (a), 6 h polymerization and 64kU/L Klenow polymerase (b), 12 h polymerization and 64 kU/L Klenow polymerase (c).(d) Statistical analysis of the content of the dimer-origami tiles after 6-hour polymerization with 32 kU/L Klenow polymerase at 37℃ (blue: *n*=36, 89%), after 6-hour polymerization with 64kU/L Klenow polymerase at 37℃ (orange: *n*=42, 71%), and after 12-hour polymerization with 64 kU/L Klenow polymerase at 37℃ (red: *n*=41, 21%), respectively. Scale bars, 400 nm.

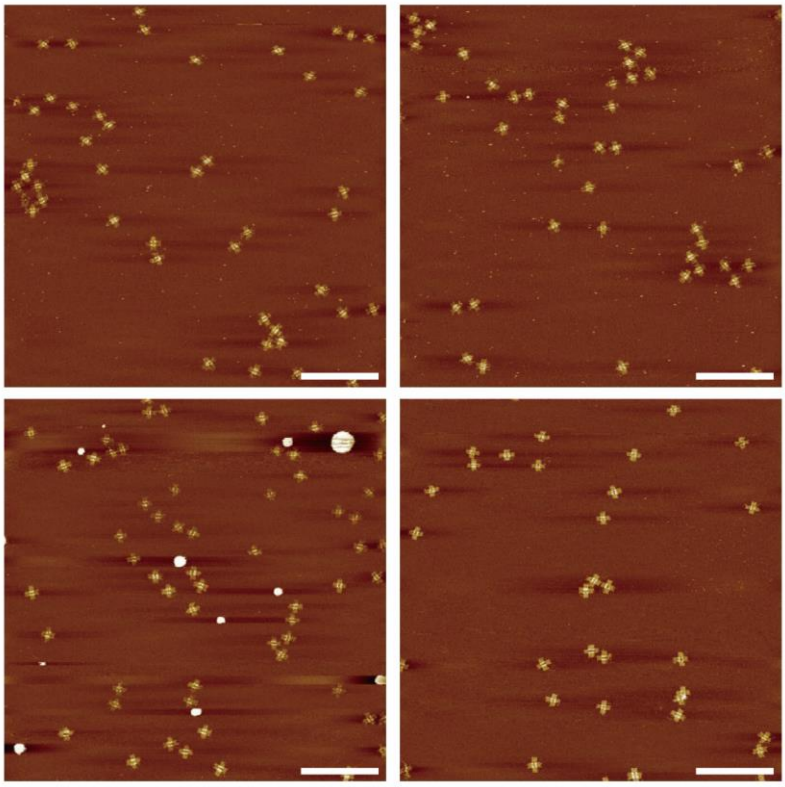

Figure S13. AFM image of the origami dimers after treatment with the primer strand “cab” and 64 kU/L Klenow polymerase, incubating for 12 h at 37°C. Scale bars, 400 nm.

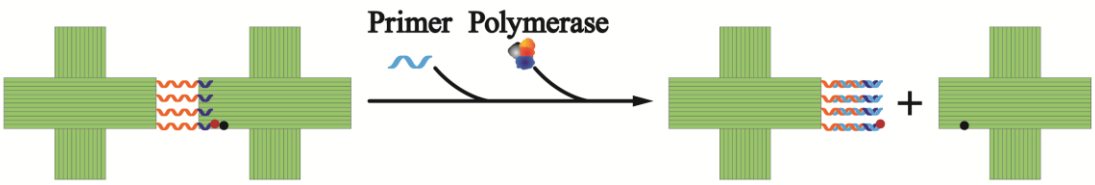

Figure S14. Schematic of the disassembly of the quencher (BHQ1, black dot) and fluorophore (6-FAM, red dot) modified DNA origami dimer based on polymerase-triggered strand displacement.

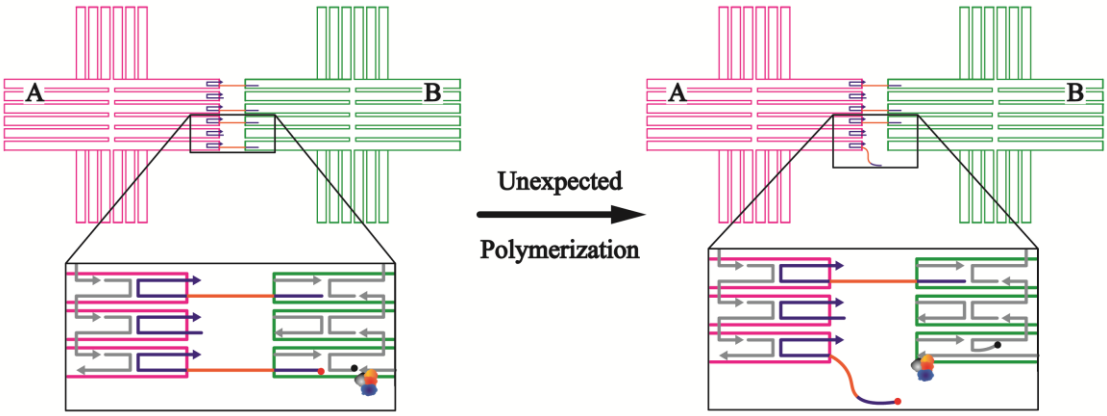

Figure S15. Schematic of the unexpected polymerization. The quencher (BHQ1, black dot) and fluorophore (6-FAM, red dot) were modified on the DNA origami dimer. The duplex at the edge of the DNA origami initiated polymerase-triggered strand displacement, resulting in the removal of the fluorescent-modified staple from the origami structure. This caused the separation of the quencher and fluorophore.

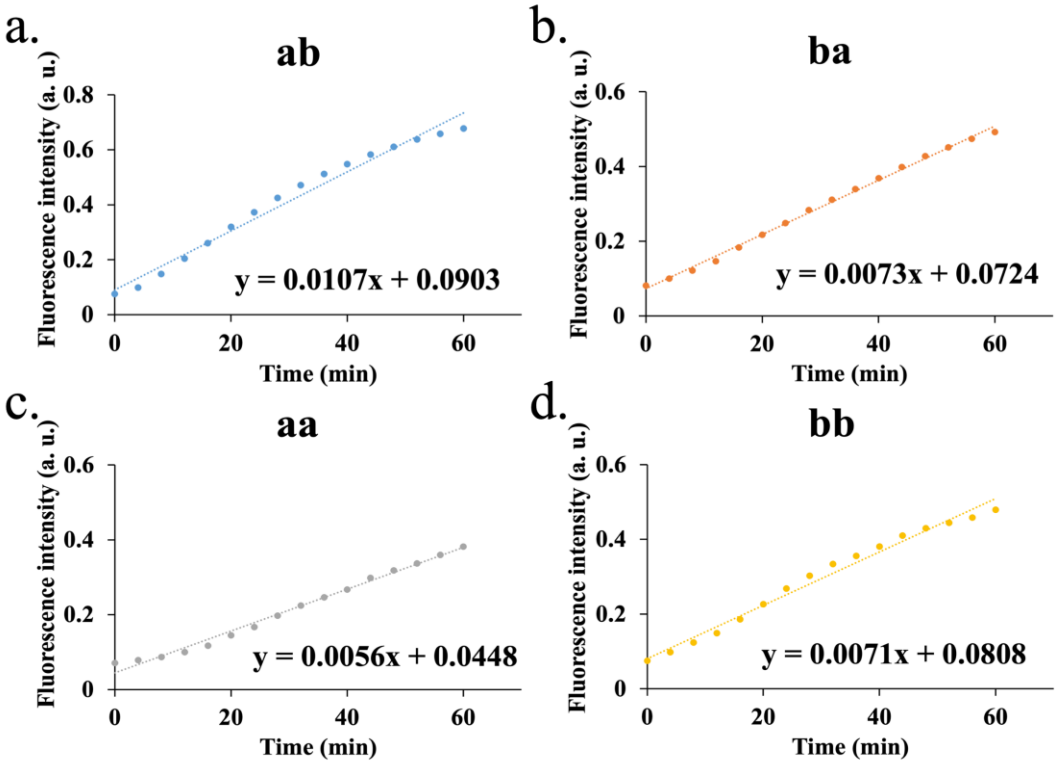

Figure S16. Initial rates of different primers addition systems.

To estimate the dissociation rate constants of the disassembly systems, we calculate the initial rate for the first 60 min of the fluorescence intensity recovery curves by fitting the curve slope in the initial states in each primers addition system shown in Figure 3e.

Compared to the reaction systems with primer “ab” (system “ab”) and primer “ba” (system “ba”), the system “ab” has a faster initial rate. We guess that the polymerization reaction starting with primer “ba” undergoes an additional 7-nt-long region than the polymerization reaction starting with primer “ab” (Figure 3a). In the system “bb”, the polymerase reacts preferentially with the duplex without overhang at the 3' end. Therefore,

the initial rate of the system “bb” is roughly the same as that of the system “ba”. In the system “aa”, we speculate that some possible complexes, such as the three-strand structure, make the rate of the polymerization reaction lower than the former three systems.

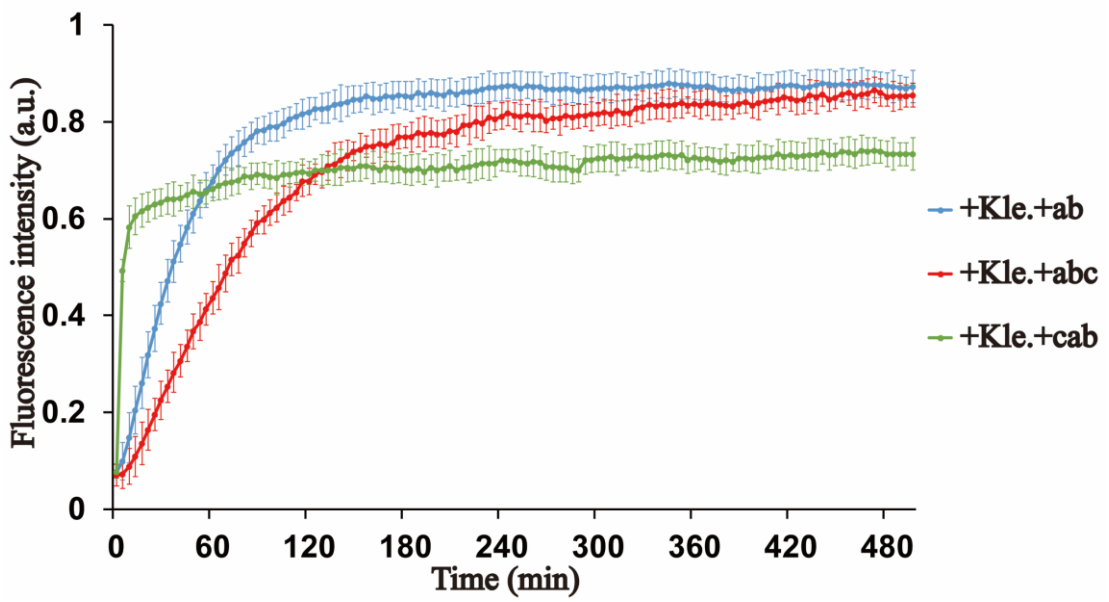

Figure S17. The melting curve of the polymerase-triggered disassembly strategy using the primers containing overhang. The error bars stand for the standard deviations.

In the presence of Klenow and primers “abc”, a significant increase in fluorescence was observed. The fluorescence intensity was close to the sample with primers “ab”. The fluorescence curve of the sample with primer “cab” rose faster than that of primer “abc”, but the increase was lower. This is because, in order to stimulate the exonuclease activity of Klenow to remove the overhang at the 3' end of primer “cab”, the sample requires a higher concentration of Klenow polymerase (64kU/L) than the other samples. These results indicated that the overhang at the 3' end of the primer strand hindered the origami dimers disassembly, while the overhang at the 5' end of the primer strand had no influence on the origami dimers disassembly. Of note, to visually compare the effect of primers with and

without overhang on the disassembly efficiency, the blue curve shown in this figure is identical to that shown in Figure 3e.

To capture the upward slope of the fluorescence curve in the initial state, we added 8 kU/L of Klenow to the samples for the fluorescence experiments to reduce the reaction rate. However, the AFM results showed that the low concentration of Klenow hardly disassembled the origami dimer in the system “cab”. So, we used 64kU/L of Klenow for the fluorescence experiments of system “cab”. An 8-fold excess of polymerase makes the initial rate of system “cab” much higher than that of other systems. In addition, we believe that the higher polymerase concentration also caused a more intense leakage reaction (Figure S15), which further increased the reaction rate of system “cab”.

It is worth mentioning that the fluorescence intensity of system “cab” is lower than system “ab” and system “abc” at the steady state, which is consistent with the results of the AFM experiment. We believe that the efficiency of Klenow is not 100%, neither for exonuclease activity nor for polymerase activity. Therefore, we speculate that not all overhangs on the primer cab were removed. The primer cab with surviving overhang further hindered the polymerase reaction, resulting in a lower fluorescence

intensity of system “cab” than system “ab” and system “abc” at the steady state.

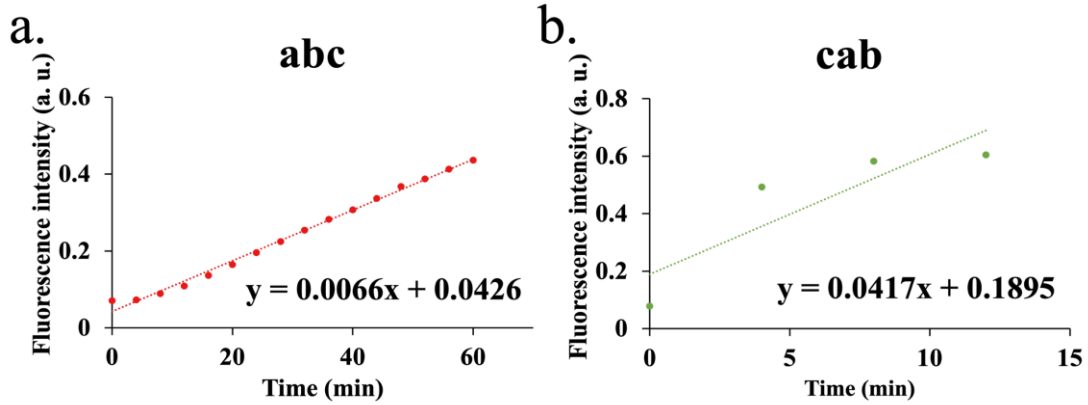

Figure S18. Initial rates of "abc" and "cab" primers addition systems.

The initial rates of system "abc" and system "cab" were calculated by fitting the curve slope of fluorescence curves at the first 60 min and 12 min, respectively. The slopes are denoted as S_{abc} and S_{cab}, respectively.

Since the polymerase concentration in the system "cab" is eight times higher than the polymerase concentration in the system "abc", to roughly compare the initial rates of the two systems under the same conditions, we compared $S_{cab}/8$ (~ 0.0052) and S_{abc} (~ 0.0066). The results illustrate that system "abc" has a faster initial reaction rate, proving it takes some time to remove the overhang.

In addition, system "ab" has a faster initial rate than system "abc". We speculate that the spatial site resistance at the DNA origami dimer junction affects the binding of primer abc to the connecting strands, thus affecting the initial reaction rate.

3. Supporting Tables

Table S1. The detailed analysis of the disassembly of the origami dimers with the primer strand and Klenow polymerase.

Description		Origamis Counted
Prior to disassembly	Singles	21
	Dimers	184(89.76%)
	In all	205
After interaction with the primer strand and the klenow polymerase, 16kU/L	Singles	175
	Dimers	28 (13.79%)
	In all	203
After interaction with the primer strand and the klenow polymerase, 32kU/L	Singles	185
	Dimers	14 (7.04%)
	In all	199

Table S2. The detailed analysis of the disassembly of the origami dimers controlled by programmable polymerase primer and Klenow polymerase.

Description		Origamis Counted
Prior to disassembly	Singles	15
	Dimers	184(92.46%)
	In all	199
After interaction with the klenow polymerasethe and the primer strand “ab”	Singles	179
	Dimers	16 (8.21%)
	In all	195
After interaction with the klenow polymerasethe and the primer strand “ba”	Singles	181
	Dimers	18 (9.05%)
	In all	199
After interaction with the klenow polymerasethe and the primer strand “aa”	Singles	52
	Dimers	148(74.00%)
	In all	200
After interaction with the klenow polymerasethe and the primer strand “bb”	Singles	49
	Dimers	152(75.62%)
	In all	201
After interaction with the klenow polymerasethe and the primer strand “abc”	Singles	182
	Dimers	18 (9.00%)
	In all	200
After interaction with the klenow polymerasethe and the primer strand “cab”	Singles	155
	Dimers	42 (21.32%)
	In all	197

Table S3. Thermal annealing ramp for DNA origami.

Temperature[℃]	Duration[min/℃]
90	0.5
86-71	1
70-60	10
59-30	15
29-26	10
24	forever

Table S4. Oligonucleotide sequences of the staple strands for the cross-shaped DNA origami tiles:

Name	Sequence (from 5′ to 3′)
CO-1	ATAATATCCCATCCTAATTTACGATTCCTTAT
CO-2	TATCAACAATAGATAAGTCCTGAAAAGTACCG
CO-3	AACATGTTCAGCTAATGCAGAACGTTTTTATT
CO-4	ATTCTGTCCAGACGACGACCCAATAG
CO-5	AATTGAGAATCGCCATATTTAACAATACCGAC
CO-6	CAGTATAAAGCCAACGCTCAACAGAAGAATAA
CO-7	CATTCCAACTGAATCTTACCAACGAGTTACAA
CO-8	CACTCATCGTTGCTATTTTGCACCCAATCCAA
CO-9	TTCATCGTGCGGGAGGTTTTGAAGGTCAAAAA
CO-10	CAAGCAAAAACGCGAGGCGTTTTAGAATAACA
CO-11	TTTTTCAAGCAAGACAAAGAACGCTATCCGGT
CO-12	CTGACCTAAACTATATGTAAATGCTTTTTAAT
CO-13	CGTGTGATCTTTTTAACCTCCGGCTGTGAGTG
CO-14	ACACCGGAAATTTATCAAAATCATCGCTATTA
CO-15	ATTTTATCGAACGGGTATTAAACCCAAGAAAA
CO-16	CAAGATTAGAGAACAAGCAAGCCGCGCCTGTT
CO-17	ATTCTAAGTCAGATATAGAAGGCTGAGAAAAC
CO-18	ATCCAATCATATATTTTAGTTAATCGAGCCAG
CO-19	GAGACTACAAATAAGGCGTTAAATTAGGGCTT
CO-20	CAATAGTGATCATAATTACTAGAAATTCTTAC
CO-21	AATAAACAAAATAGCAATAGCTATGATAGCCG
CO-22	ATAAGAAAGTTAAGCCCAATAATAAGGAAACG
CO-23	TGAAAATACTAATATCAGAGAGATAACTGGCATGA
CO-24	TAAAAACACACCCTGAACAAAGTCAGAGGGTA
CO-25	TTAACAATTCAAGAAAACAAAATTGGGAGAAT
CO-26	GGAAACAGTACCTGAGCAAAAGAAGATGATGA
CO-27	AATAACCTAAATCGCGCAGAGGCGCTTAGCCG
CO-28	ATTAATTTATTCGCCTGATTGCTTGAAATTGC
CO-29	AAACAATGGCCATATTATTTATCCCAGCTACA
CO-30	AGAATTGACGATTTTTTGTTTAACCCTTAAAT
CO-31	ATTGAGCGGCAGCCTTTACAGAGAGCGAACCT
CO-32	TAACTGAAGGGAAGCGCATTAGACAATTACAT
CO-33	ATTTCAATTACATAAATCAATATATTAGGTTG
CO-34	AAGTTACATGCTTCTGTAAATCGTAGGTCTGA
CO-35	AATAACGGTCCCTTAGAATCCTTGAGAAGAGT
CO-36	AACAAAGTTCGCACTCCAGCCAGCGCAAAGCG
CO-37	CAATAATAAGGGGACGACGACAGTCTGTTGGG
CO-38	TGCTCCATGTTAAATTATTC

CO-39	GAACGAGGCGCAGACAGAAATAAATGAATACC
CO-40	GTAGATTTACCATATCAAAATTATGATTATCA
CO-41	CAGGAAGATACCAGAAGGAAACCGAGAGCAAG
CO-42	CCAGTTTGACGGAATACCCAAAAGAACCCACA
CO-43	GCATCGTAACCGCGCTATTA
CO-44	TTGCACGTAAAACGGTCAATCATAAGGGAACCACCAGAAG
CO-45	TAGAACCTTCAGGTTTAACGTCAGGGAGAAAC
CO-46	CCATTCGCGCTGTTTCCTGTGTGAACGAGCCG
CO-47	AAGGGCGAGTACCGAGCTCGAATTGTGCCTAA
CO-48	CGCCAGCTATGCCTGCAGGTCGACGCGTTGCG
CO-49	ACGTTATTACTCGTATTAAATCCTGTTTTCCC
CO-50	ATCATTTTTAGAAGTATTAGACTTCCTGCAAC
CO-51	GAGCGGAAAGAGCCGTCAATAGATAAATGAAA
CO-52	GATGATGGATATCTTTAGGAGCACCCTCAAAT
CO-53	TGGTCATACATTCAGGCTGCGCAAATCGGCCT
CO-54	ATCCCCGGTCGGTGCGGGCCTCTTTGCATCTG
CO-55	AAGCTTGCGGCGAAAGGGGGATGTGCTGCAAG
CO-56	AGTCACGAGTTGGGTAACGCCAGGTTGCCCGA
CO-57	ATTCGACAAATTTTAAAAGTTTGAGTAACATT
CO-58	TGAGGATTGCGGAACAAAGAAACCGAACTGACCAA
CO-59	AATAGATTTTATCATCATATTCCT
CO-60	TATCTAAACAATTCATCAATATAAGGAAGGGT
CO-61	GAAGCATAGCAGCAAGCGGTCCACTGATGGTG
CO-62	TGAGTGAGATTGCCCTTCACCGCCATAAATCA
CO-63	CGTGCCAGAGGCGGTTTGCGTATTATTAAAGA
CO-64	GTGAGGCGCCACCAGCAGAAGATAGCCAACGC
CO-65	AATCTAAAGCTATTAGTCTTTAATCTCAATCG
CO-66	ATCAAACCAGAATACGTGGCACAGCAGATTCA
CO-67	AGAGAGTTAAGTGTAAAGCCTGGGCGTAATCA
CO-68	AACAGCTGCTAACTCACATTAATTTCTAGAGG
CO-69	GGGTGGTTCCGCTTTCCAGTCGGGCCAGTGCC
CO-70	GCGGGGAGCTGCATTAATGAATCGAAACAGAG
CO-71	CGAACGAAGTCAGTATTAACACCGTACAAACA
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CO-73	TTTGAATGGCATCACCTTGCTGAATAACAACT
CO-74	AAAGCGTACTCAATCAATATCTGGAGGAAGGT
CO-75	GTTCCGAAATCGGCAAAATCCCTTTGGCCCTG
CO-76	AAAGAATAGCCCGAGATAGGGTTGAGACGGGC
CO-77	CATGGAAATACCTACATTTTGACGGCGCGAAC
CO-78	TCTGAAATGGATTATTTACATTGGACAATATT
CO-79	CCAGTCACACGACCAGTAATAAAACTGACCTG
CO-80	CAGCTTTCAATTCGCATTAAATTTATGCCGGA
CO-81	ACAACCCGTATTTAAATTGTAAACTACAAAGG

CO-82	GCGGATTGAGCCCCAAAAACAGGATGGAGCAA
CO-83	TATGTTAGCAAACGTAAGCAAAATCACCAGTAACCAATGA
CO-84	ACATATAATCACCGACTTGAGCCACAGTAGCG
CO-85	TAAGTTTAGGAAATTATTCATTAATCAGACTG
CO-86	CATATGGTAACCGATTGAGGGAGGGGTCATAG
CO-87	TCAGCTCAATTCGCGTCTGGCCTTCCTGTAGC
CO-88	TTTGTTAAATCAACATTAAATGTGAGCGAGTA
CO-89	ACCATTAGCAATCATATGTACCCCCGTAAAAC
CO-90	TATCACCGAAGAAACGCAAAGACACCACGGAA
CO-91	ATATTGACTTTTGTCACAATCAATAGAAAATT
CO-92	GAGGGTAGAAGATTCAAAAGGGTGGCATTAAC
CO-93	CTATCAGGTTTAAATGCAATGCCTCAAAGAAT
CO-94	ACAAGAGACAAGGATAAAAATTTTCAGAGCAT
CO-95	TAGCATGTCAAGGCCGGAAACGTCGCACCATT
CO-96	AACCATCGACCACCACCAGAGCCGAGACGATT
CO-97	ACAGAATCTCAGAGCCACCACCCTAATCCTCA
CO-98	TAGCGCGTCCTCAGAGCCGCCACCTCTCTGAA
CO-99	CCCCCTTAACCGGAACCAGAGCCACATGGCTT
CO-100	CGGAGACATCTAGCTGATAAATTATTGTTAAA
CO-101	TGTAGGTACTATTTTTGAGAGATCGTTAATAT
CO-102	TCATATATTCATTGCCTGAGAGTCAGATTGTA
CO-103	ATTGACAGATACTTTTGCGGGAGAACATTATG
CO-104	CGCCACCCAAGTTTGCCTTTAGCGAGGTGAAT
CO-105	ACCGCCTCTTTCATCGGCATTTTCGAAGGTAA
CO-106	ATCCAATAAATGGTCAATAACCTGGGATTAGA
CO-107	TAGCAAAAAGATTTAGTTTGACCAATAAGAGG
CO-108	AAAGCTAAATAACAGTTGATTCCCAATTCT
CO-109	ACCCTGTAGAGGTTGAGGCAGGTCCCGCCAGC
CO-110	GGCCTTGAAGACTCCTCAAGAGAAAGGCGGAT
CO-111	TTTACCGTCAGTTAATGCCCCCTGTCAGGAGG
CO-112	TTGATGATGTCAGTGCCTTGAGTAGCCACCCT
CO-113	TATTTTCATCTACTAATAGTAGTAAGAAAGGC
CO-114	ATTTCGCAAATCATACAGGCAAGGGAGTAATG
CO-115	ATTAGCGGCTAAAGTACGGTGTCTTGTTTTAA
CO-116	AGAGGCTGTATTCACAAACAAATACAGAGCCG
CO-117	GGAACCTAAGAATGGAAAGCGCAGCTCAGAAC
CO-118	CGTATAAATCCAGTAAGCGTCATACCACCGGA
CO-119	GAGTACCTGAAGCCCGAAAGACTTGCAAAAGA
CO-120	TCATTTTTAGCAAAGCGGATTGCAAAAATGTT
CO-121	TGAATATAATCAGGTCTTTACCCTCGGAATCG
CO-122	ATATGCAAGGTTTTGCTCAGTACCGGATTAGG
CO-123	TTTAGTACAACACTGAGTTTCGTCGGAACAAC
CO-124	CAGAACCGGATAGCAAGCCCAATATTTCACGT

CO-125	GCGTTTTAAAACTCCAACAGGTCATTTAGCTA
CO-126	ATTAAGAGTTAATTGCTCCTTTTGTTAGATAC
CO-127	AATCAAAAATGCTGTAGCTCAACAGGAAGTTT
CO-128	GTCGTCTTGTTCAGAAAACGAGAATCAAATGC
CO-129	CCCTCATATCGAGAGGGTTGATATAAGTAT
CO-130	AAACTACAAGGTGTATCACCGTACCCTATTTC
CO-131	TGTACCGTCGCCACCCTCAGAACCACAGTGCC
CO-132	AGTTTTGCTACGAGGCATAGTAAGAGATGGTT
CO-133	TAGACTGGAACTAATGCAGATACAATTACCTT
CO-134	TTTAAACATCCAGACGTTAGTAAAAAAGTTTT
CO-135	GTTTCAGCGCCGACAATGACAACAAGCATCGG
CO-136	TAAAGGAAAATTTCTTAAACAGCTGGCTTTGA
CO-137	TGAAAATCTTGTATCGGTTTATCAAGTTTCCA
CO-138	TATCATAAATAGCGAGAGGCTTTTCAAATATC
CO-139	AAAGGAATCAGAGGGGGTAATAGTTCAAAAAG
CO-140	CCACATTCATAGCGTCCAATACTGGACTATTA
CO-141	TAGAAAGAATTCATTGAATCCCCCTGACCATA
CO-142	CTTGCAGGCTAACGGAACAACATTACGTTAAT
CO-143	CCACGCATGATTTTGCTAAACAACACAGACAG
CO-144	ATAGTTGCGGAGTGAGAATAGAAAACCAGTAC
CO-145	TCGAGGTGTTGCGAATAATAATTTGGAACCCA
CO-146	TAATTTCATCAACGTAACAAAGCTGCTCATTC
CO-147	ATGCGATTTCTTGACAAGAACCGGATATTCAT
CO-148	AAAACGAAGAGTTAAAGGCCGCTTCGCTGAGG
CO-149	GGACTAAAAAACACTCATCTTTGACCCCCAGC
CO-150	TTAAACGGACCTAAAACGAAAGAGGCAAAAGA
CO-151	AGTGAATATAGTAAATTGGGCTTGAGCAACAC
CO-152	TACCCAAAACTTTAATCATTGTGATAACGCCA
CO-153	AAGAGTAATTAAGAACTGGCTCATTAGGAATA
CO-154	CGGTGTACAGACCAGGTTGGGAAGAAAAATCTATTACAGG
CO-155	AGATTTGTCTCAGCAGCGAAAGACACCATCGC
CO-156	GATTATACTAGCAACGGCTACAGATGATACCG
CO-157	ATACACTAGACTTTTTCATGAGGAGCTTGCTT
CO-158	TACCGACAATTGAAAGTATTA
CO-159	TAATAAGAGAATATAAAGTTAGCCCGGAATACGCCTGTAGCATTCCTTTCAACA
CO-160	CCCGACTTAGGAATCATTTAGAGCCGAAAATACATACATAAAGGTGGCA
CO-161	GGTTATATAATTTAATGGTTTGAAACGCCAACATGTATAAGTACAACGG
CO-162	AACAAACATTCATTTGTCTGTATGGAACCGATATATTCGGTTTGCGGGA
CO-163	GCGATTAACGTTGTAATTTTCAACGATCGATGAACGGTAATGGTTGATA
CO-164	CTCACTGCTTTCTTTTCACCAGTGAGTGTTGTTCCAGTGGAACAAACG
CO-165	AGTGCCACCTAAAACATTCAGGACGCGCATAGGCTGGCTGACCTTCATC
CO-166	ACGTGGACTCCAACGTCTTGCGAACGAGTTTAAGCAATAAAGCCTTAGAACCC
CO-167	AAAGGGCGAATAGCTTAATTGC

CO-168	TAAGCAAATCGGATTCTCCGTGTTTTGGAACAAGAGTCCACTGGGCGCCA
CO-169	ATCAGAAAACCGTAATGGGATAGGTCACGTTGGTGTAGATTTACTCCTTATTACGCAG
CO-170	CCACCAGAATAGCAGCACCGTAATTTTGGGAATTTACCGCGCAATAAAC
CO-171	TTAAAGCCTTATTCTGAAACATAAGGTAAAGTA
CO-172	CATTCCATATCGGTTGTACCAAAAAGCCTTTATAACGACGGAAACCTGT
CO-173	AAGTGCCGGTTAGCGTAACGATCTTGAATTTTTAATTACCTTGATGCAA
CO-174	TAGTCAGAGCGGATGGCTTAGTAAACCGTCTATCAAGCCATTGCAACAGGAAAAAATAC
CO-175	TCATAAATTTCATCAGTTGAGATTTATACCAGTTCGCCATTAAAACGCT
CO-176	TCGTCACCATCATCGCCTGATAAATTGTGTCGAAATCCGCTTAAAGAGGACAGATGAA
CO-177	AACGAGGGCAAGCGCGAAACATATTTAGGCAGAGGCATTTTTTCATCTT
CO-U-1	TTTTCAATAATCGGCTGTCTGCATGTAGAAACCAATTTTT
CO-U-2	TTTTCAGAGCCTAATTTGCCCTAACGAGCGTCTTTCTTTT
CO-U-3	TTTTTTAAGAAAAGTAAGCACTTACCGAAGCCCTTTTTTT
CO-U-4	TTTTTGGTGCCGGAAACCAGTTTCCGGCACCGCTTCTTTT
CO-U-5	TTTTCAATTCCACACAACATAATTGTTATCCGCTCATTTT
CO-U-6	TTTTAGGCGAAAATCCTGTTGCTGGTTTGCCCCAGCTTTT
CO-D-1	TTTTCATATGCGTTATACAAAAAGCCTGTTTAGTATTTTT
CO-D-2	TTTTTTAGATTAAGACGCTGAAAACATAGCGATAGCTTTT
CO-D-3	TTTTAGTACCTTTTACATCGATGAATATACAGTAACTTTT
CO-D-4	TTTTTATACTTCTGAATAATTCCTGATTGTTTGGATTTTT
CO-D-5	TTTTCAGTTGAAAGGAATTGTCAGTTGGCAAATCAATTTT
CO-D-6	TTTTCAGAGATAGAACCCTTGGGACATTCTGGCCAATTTT
CO-L-1	TTTTAACGCCATCAAAAATATTTTTTAACCAATAGGTTTT
CO-L-2	TTTTTATGATATTCAACCGTGTCAAATCACCATCAATTTT
CO-L-3	TTTTAAAAGGTGGCATCAATTTTGGGGCGCGAGCTGTTTT
CO-L-4	TTTTGAACCAGACCGGAAGCATTCGAGCTTCAAAGCTTTT
CO-L-5	TTTTGACGATAAAAACCAAACCCTCGTTTACCAGACTTTT
CO-L-6	TTTTGAAACACCAGAACGAGAGGCTTGCCCTGACGATTTT
CO-R-1	TTTTCAAAAGGGCGACATTCTTACCAGCGCCAAAGATTTT
CO-R-2	TTTTTTTCATAATCAAAATCTTAGCGTTTGCCATCTTTTT
CO-R-3	TTTTAATAAGTTTTAACGGGACAGGAGTGTACTGGTTTTT
CO-R-4	TTTTCACCCTCATTTTCAGGCCACCCTCAGAGCCACTTTT
CO-R-5	TTTTCAAAAGGAGCCTTTAATCCAAAAAAAAGGCTCTTTT
CO-R-6	TTTTCACTACGAAGGCACCAGTAAAATACGTAATGCTTTT

Table S5. Oligonucleotide sequences of the connecting strands:

Name	Sequence (from 5′ to 3′)
A-R-L-1	TTTTTTAACCAATAGGTTTTGGGATTATCTCCATGGGATTATTTTCAAAAGGGCGACATT CTTACCAGCGCCAAAGATTTT
A-R-L-3	TTTGGGGCGCGAGCTGTTTTGGGATTATCTCCATGGGATTATTTTAATAAGTTTTAACGG GACAGGAGTGTACTGGTTTTT
A-R-L-4	ATTCGAGCTTCAAAGCTTTTGGGATTATCTCCATGGGATTATTTTCACCCTCATTTTCAG GCCACCCTCAGAGCCACTTTT
A-R-L-6	AGGCTTGCCCTGACGATTTTGGGATTATCTCCATGGGATTATTTTCACTACGAAGGCACC AGTAAAATACGTAATGCTTTT

Table S6. Oligonucleotide sequences of the programmable primer strands:

Name	Sequence (from 5′ to 3′)
p	TAATCCCATGGAGATAATCCC
ab	ATGGAGATAATCCC
ba	TAATCCCATGGAGA
bb	ATGGAGAATGGAGA
aa	TAATCCCTAATCCC
abc	TGTGTGTATGGAGATAATCCC
cab	ATGGAGATAATCCCTGTGTGT

Table S7. Oligonucleotide sequences of the modified strands:

Name	Sequence (from 5′ to 3′)
sta_F	6-FAM-TTGGCTTGCCCTGACGATTTTGGGATTATCTCCATGGGATTATTTTCACTACGAAGG CACCAGTAAAATACGTAATGCTTTT
sta_Q	BHQ1-TTGAATATAGTAAATTGGGCTTGAGCAACAC