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This supplementary information was **updated** on

21/12/2021.

DNA nanostructures directed by RNA clamps

The authors regret that the previous ESI required some amendments, specifically to the section on 'Negative stain EM imaging' and to the captions of Figures S8-10. The original section text and captions are as displayed below for future reference:

Negative stain EM imaging.

3 μL of solution (nanostructures concentration: 30 nM) was deposited on the Quantifoil grid, and wait 60 seconds to allow samples adsorption. Blotted the grid from the side with a piece of filter paper and applied 4 μL of the buffer (1X TAE/Mg2+), immediately stained with 0.75% (w/v) uranyl formate. EM was performed using a JEM-1400 electron microscope operating at 120 KeV and equipped with an EMSIS Morada G3 device camera. Micrographs were collected at nominal magnifications of X 80000(4.95 Å /pixel). The images were acquired at a defocus value of -1.2~-2.0 μm.[5]

Fig. S8 Original Negative stain EM data of square, expanded DNA triangle. (a) EM imaging of square (40D+15R). The size of square was too small to image, it was only a light spot imaged in the EM. (b) EM imaging of expanded triangle (93D+21D+15R).

Fig. S9 Original Negative stain EM data of expanded DNA square and pentagon. (a) EM imaging of expanded square (124D+21D+15R). (b) EM imaging of expanded pentagon (155D+21D+15R).

Fig. S10 Cryo-EM imaging of (a) and (b) expanded DNA triangle (93D+21D+15R), (c) square (124D+21D+15R) and (d) pentagon (155D+21D+15R). Scale bar is 20 nm.

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Supporting Information

DNA nanostructures directed by RNA clamps

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Experimental Procedures

Oligonucleotides.

All oligonucleotides are purchased from Sangon Biotech company (Shanghai, China). Oligonucleotides are purified by HPLC or denaturing PAGE.[1, 2]

Assembly reaction.

The 10× nanostructure assembly buffer is 10× TAE/Mg^{2+} buffer containing 400 mM Tris-base, 200 mM acetic acid, 20 mM EDTA, and 125 mM magnesium acetate, pH 8.0.[3, 4] All oligonucleotides are diluted to 25 μM by dd H_2O. RNA square assembly reaction system containing 10R (4 μL), 15R (4 μL), dd H_2O (1 μL) and 10× assembly buffer (1 μL). Triangle assembly reaction system containing 30D (1 μL), 15R (3 μL), dd H_2O (5 μL) and 10× assembly buffer (1 μL). Square assembly reaction system containing 40D (1 μL), 15R/15O (4 μL), dd H_2O (4 μL) and 10× assembly buffer (1 μL). Pentagon assembly reaction system containing 50D (1 μL), 15R/15O (5 μL), dd H_2O (3 μL) and 10× assembly buffer (1 μL). The oligonucleotides assembly ratios of expanded nanostructures were shown as followed, 93D: 21D: 15R = 1: 3 :3; 124D: 21D: 15R = 1: 4 :4; 155D: 21D: 15R = 1: 5 :5. All nanostructures were assembled in 2.5 μM concentration. All assembly reaction system were annealed from 95℃ to room temperature and stay at 4℃ over 1h.

Nondenaturing PAGE.

The 12% nondenaturing gel was prepared with 37.5:1 acrylamide/bisacrylamide gel and 1× TAE/Mg^{2+} running buffer containing 40 mM Tris-base, 20mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate, pH 8.0. The 12% nondenaturing gels were running at 150V for 45 min in ice-water bath.

Denaturing PAGE.

The 12% denaturing gel was prepared with 37.5:1 acrylamide/bisacrylamide gel, 8 M urea and TBE buffer containing 89 mM Tris-Borate, 2 mM EDTA, pH 8.3. The 12%

denaturing gels were running at 180V for 25 min in a 55°C incubator.

Table S1. Sequence information of the oligonucleotides

Strands	Sequence Information
10R	5’-r(CCGGCAGCCU)-3’
10D	5’-d(CCGGCAGCCT)-3’
20D	5’-d(CCGGCAGCCTCCGGCAGCCT)-3’
30D	5’-d(CCGGCAGCCTCCGGCAGCCTCCGGCAGCCT)-3’
40D	5’-d(CCGGCAGCCTCCGGCAGCCTCCGGCAGCCTCCGGCAGCCT)-3’
50D	5’-d(CCGGCAGCCTCCGGCAGCCTCCGGCAGCCTCCGGCAGCCTCCGGCAGCC T)-3’
60D	5’-d(CCGGCAGCCTCCGGCAGCCTCCGGCAGCCTCCGGCAGCCTCCGGCAGCC T CCGGCAGCCT)-3’
15R	5’-r(CCGGAGGAACUACUG)-3’
15O	5’-2’OMe-(CCGGAGGAACUACUG)-3’
15D	5’-d(CCGGAGGAACTACTG)-3’
P10D	5’-p-d(CCGGCAGCCT)-3’
P30D	5’-p-d(CCGGCAGCCTCCGGCAGCCTCCGGCAGCCT)-3’
P40D	5’-p-d(CCGGCAGCCTCCGGCAGCCTCCGGCAGCCTCCGGCAGCCT)-3’
P50D	5’-p-d(CCGGCAGCCTCCGGCAGCCTCCGGCAGCCTCCGGCAGCCTCCGGCAGC CT)-3’
21D	5’-d(CGACTCAGGTTTGACGTACGT)-3’
93D	5’-d(CCGGACGTACGTCAAACCTGAGTCGCAGCCTCCGGACGTACGTCAAACCT GAGTCGCAGCCTCCGGACGTACGTCAAACCTGAGTCGCAGCCT)-3’
124D	5’-d(CCGGACGTACGTCAAACCTGAGTCGCAGCCTCCGGACGTACGTCAAACCT GAGTCGCAGCCTCCGGACGTACGTCAAACCTGAGTCGCAGCCTCCGGACGTACGT CAAACCTGAGTCGCAGCCT)-3’
155D	5’-d(CCGGACGTACGTCAAACCTGAGTCGCAGCCTCCGGACGTACGTCAAACCT GAGTCGCAGCCTCCGGACGTACGTCAAACCTGAGTCGCAGCCTCCGGACGTACGT CAAACCTGAGTCGCAGCCTCCGGACGTACGTCAAACCTGAGTCGCAGCCT)-3’

Ligation of folded triangle, square and pentagon.

A total of 10 μL square reaction system containing P10D (100 μM), 15R (100 μM) and 1× Assembly buffer. 10 μL DNA triangle reaction system containing P30D (100 μM), 15R (300 μM) and 1× assembly buffer. 10 μL DNA square reaction system containing P40D (100 μM), 15R (400 μM) and 1X assembly buffer. 10 μL DNA pentagon reaction system containing P50D (100 μM), 15R (500 μM) and 1× assembly buffer. These reaction systems were annealed from 85°C to 4°C over 1 h. and then added 5× T4 DNA ligase buffer (4 μL), 5 U/μL T4 DNA ligase (4 μL) and dd H_2O (2 μL) to each 10 μL reaction systems, respectively, and incubated at 25°C over 6 h.

ESI-MS detection.

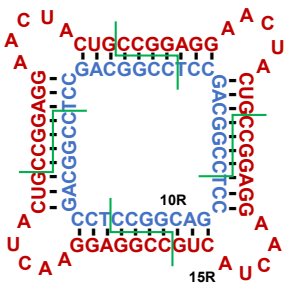
The folded triangle, square and pentagon with ligation were purified by Phenol: chloroform: isoamyl alcohol method. The purified samples were detected by ESI-MS supported by Shanghai Sangon Co. Lid.

Negative stain EM imaging.

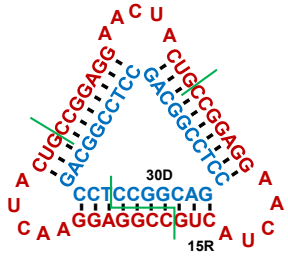
Three drops of 20 μL, 20 μL, and 60 μL 0.75% uranium formate (UF) stain solution were applied on the parafilm (Bemis). Then, 3 μL of solution (nanostructures concentration: 30 nM) was applied on the glow-discharged (40 s) 300-mesh-Cu grids (Quantifoil) coated with a continuous carbon film and waited 60 s to allow sample adsorption. The grid was blotted from the side with a piece of filter paper and washed with 4 μL of washing buffer containing 40 mM Tris-base, 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate, pH 8.0. Excessive washing buffer was blotted with filter paper and the grid was stained in the first two drops of UF followed by blotting with filter paper. The grid was stained in the third drop of UF for 40 s. Excessive UF was blotted and the grid was air-dried and stored until imaging. The grid was placed on a side-entry holder and loaded into a JEM-1400 operated at 120 kV, condenser lens aperture 150 μm, spot size 1. Micrographs were collected using RADIUS software on a Morada G3 direct electron camera under magnification of 150,000× or 250,000× (corresponding to a calibrated sampling of 2.56 Å or 1.52 Å per physical pixel, respectively).

Cryo-EM imaging

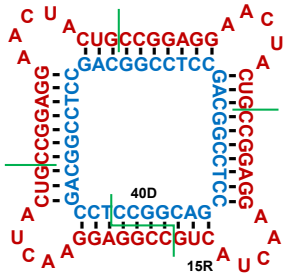
A total of 3 μL sample was applied onto glow-discharged (40 s) 200-mesh R2/1 Quantifoil Cu grids. The grids were blotted for 3 s in 100% humidity with no blotting offset and rapidly frozen in liquid ethane using a Vitrobot Mark IV (Thermo Fisher). The frozen grids were loaded into a Titan Krios (Thermo Fisher) operated at 300 kV, condenser lens aperture 50 μm, spot size 5, parallel beam with illuminated area of 1.07 μm in diameter. Microscope magnification was at 165,000× (corresponding to a calibrated sampling of 0.85 Å per physical pixel).



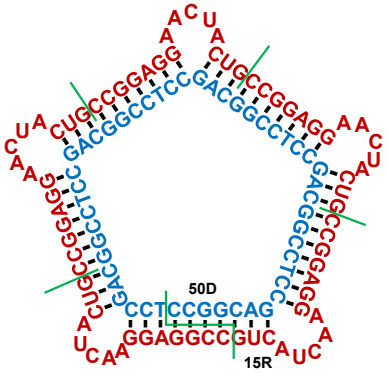
DNA square (10D+15R)



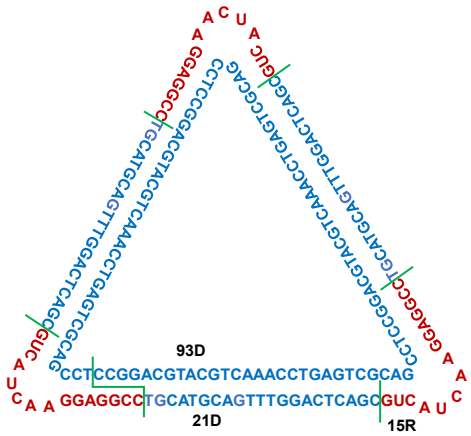
DNA triangle (30D+15R)



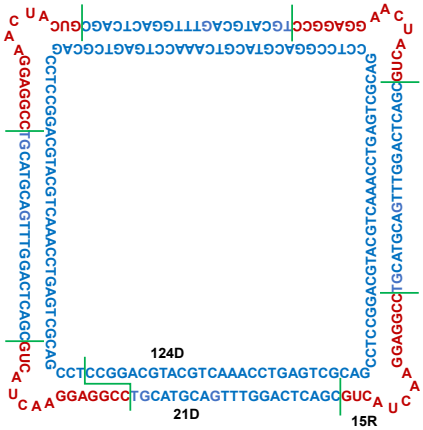
DNA square (40D+15R)



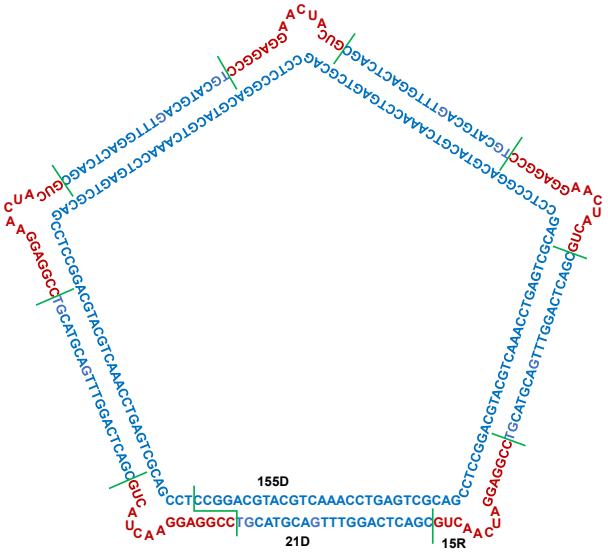
DNA pentagon (50D+15R)



Expanded triangle (93D+21D+15R)



Expanded square (124D+21D+15R)



Expanded pentagon (155D+21D+15R)

Fig. S1 Second structure information of nanostructures folded by 15R clamp.

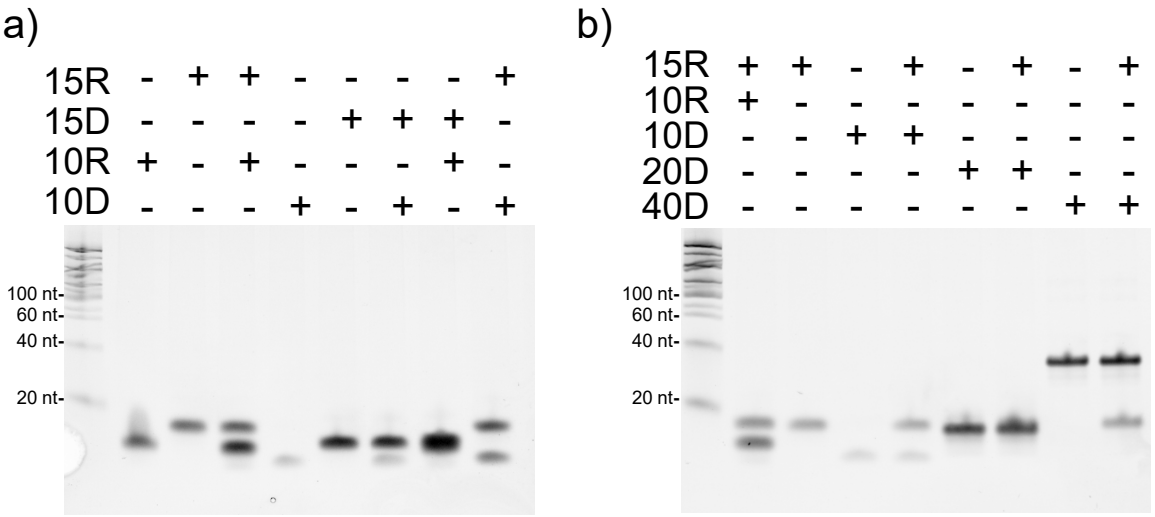


Fig. S2 Denaturing PAGE analysis of the Fig. 2.

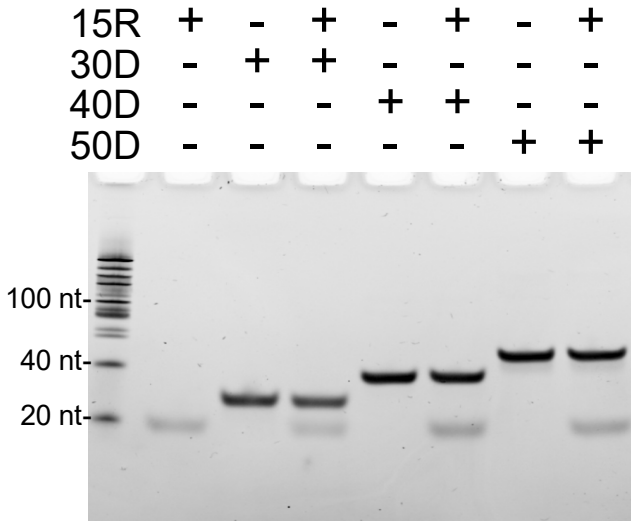


Fig. S3 Denaturing PAGE of DNA triangle, square and pentagon folded by 15R clamp.

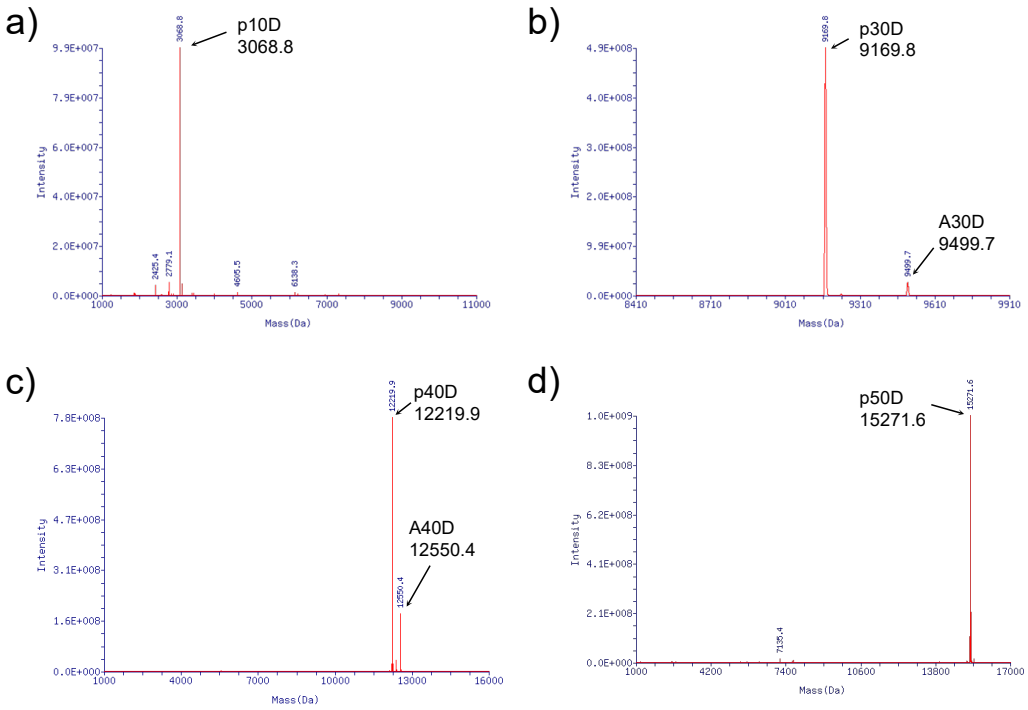


Fig. S4 ESI-MS data of the ligation with these phosphorylated scaffolds (P10D, P30D, P40 and P50D) without 15R clamp.

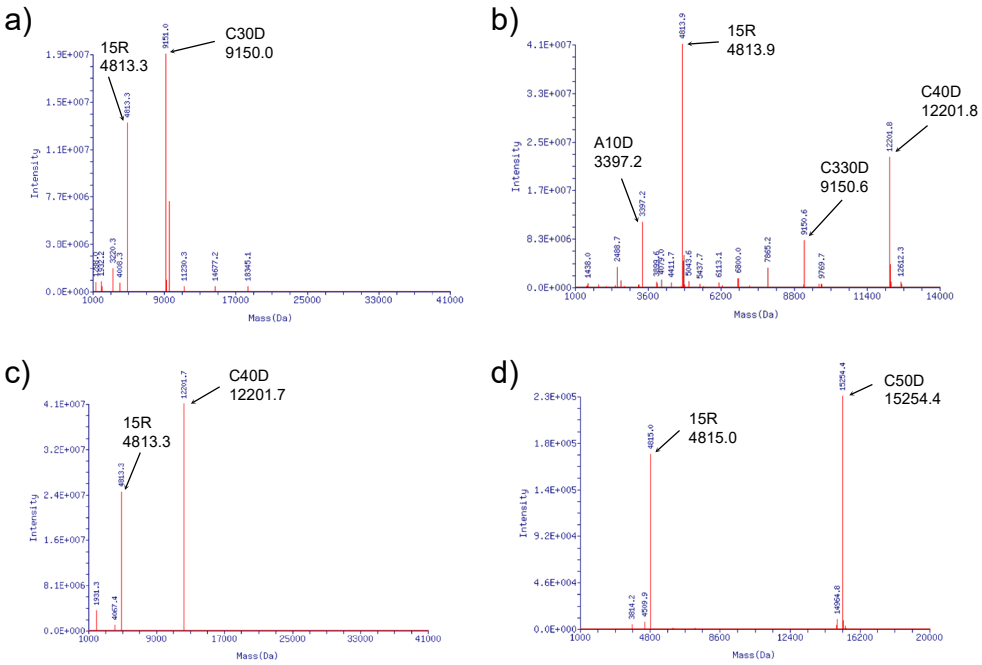


Fig. S5 ESI-MS data of (a) folded DNA triangle (P30D+15R), (b) DNA square (P10D+15R), (c) DNA square (P40D+15R) and (d) DNA pentagon (P50D+15R) ligated by T4 DNA ligase.

Tab. S2 ESI-MS data oligonucleotides

Strands	Molecular formula	Calculated $[M-H^+]^-$	Measured $[M-H^+]^-$
15R	$C_{144}H_{180}N_{61}O_{101}P_{14}$	4812.9	4813.3
P10D	$C_{95}H_{123}N_{37}O_{61}P_{10}$	3067.5	3068.8
P30D	$C_{285}H_{365}N_{111}O_{181}P_{30}$	9168.5	9169.8
P40D	$C_{380}H_{486}N_{148}O_{241}P_{40}$	12219.0	12219.8
P50D	$C_{475}H_{607}N_{185}O_{301}P_{50}$	15269.5	15271.6
A10D	$C_{105}H_{136}N_{42}O_{67}P_{11}$	3397.5	3397.2
A30D	$C_{295}H_{378}N_{116}O_{187}P_{31}$	9498.5	9499.7
A40D	$C_{390}H_{499}N_{153}O_{247}P_{41}$	12549.0	12550.4
A50D	$C_{485}H_{620}N_{190}O_{307}P_{51}$	15599.1	-
C30D	$C_{285}H_{364}N_{111}O_{180}P_{30}$	9151.8	9151.0
C40D	$C_{380}H_{485}N_{148}O_{240}P_{40}$	12202.8	12201.8
C50D	$C_{475}H_{606}N_{185}O_{300}P_{50}$	15252.5	15254.4

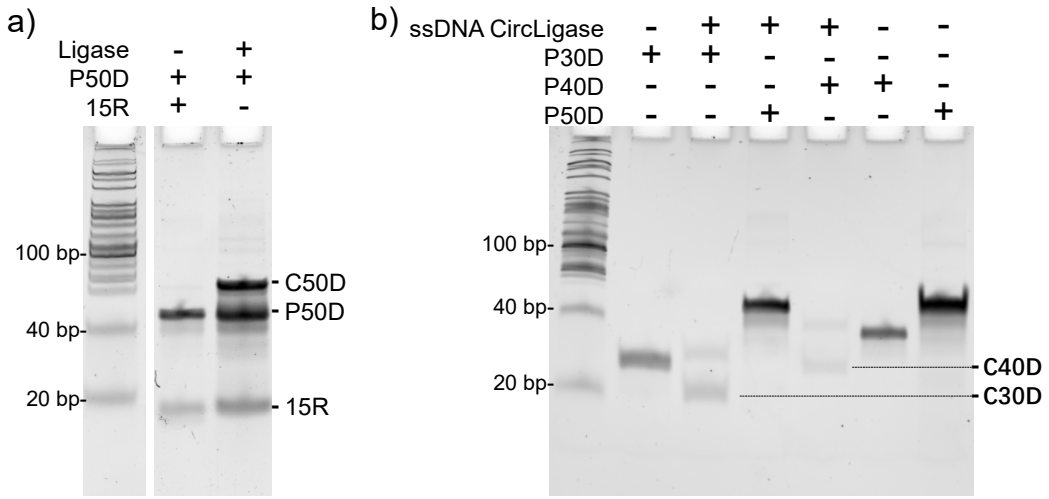


Fig. S6 Cyclized DNA detected by denaturing PAGE. (a) Denaturing PAEG analysis of assembled Pentagon (P50D+15R), Cyclized P50D (C50D) was shown on slower migration band. (b) P30D, P40D and P50D were cyclized by ssDNA CircLigase. Because of the sequence of these DNA scaffolds, the cyclization was in a low efficiency. And the P50D even couldn't cyclized by ssDNA CircLigase.

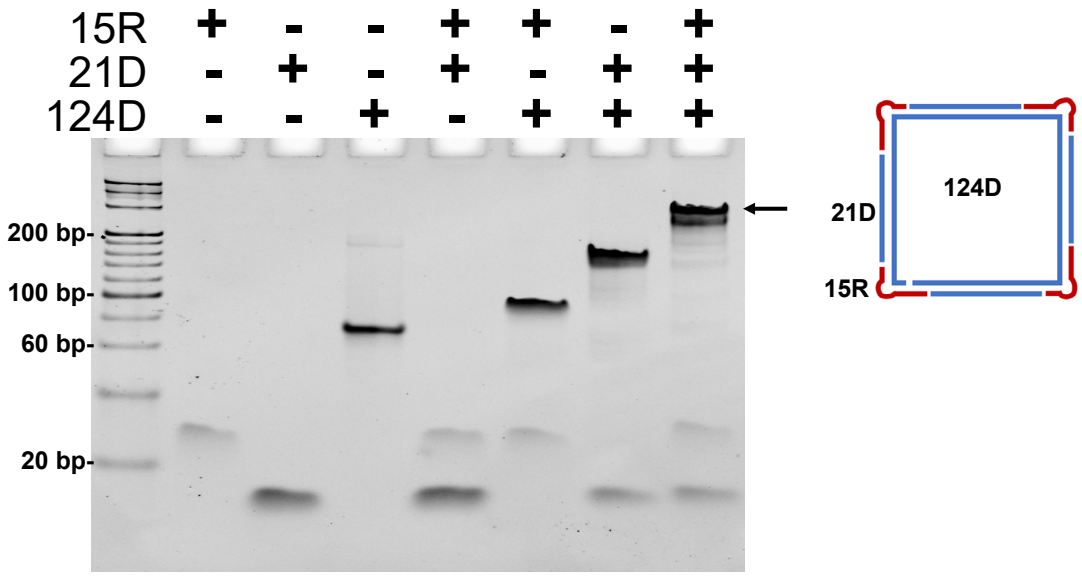


Fig. S7 Detailed assembly of expended DNA square detected by nondenaturing PAGE.

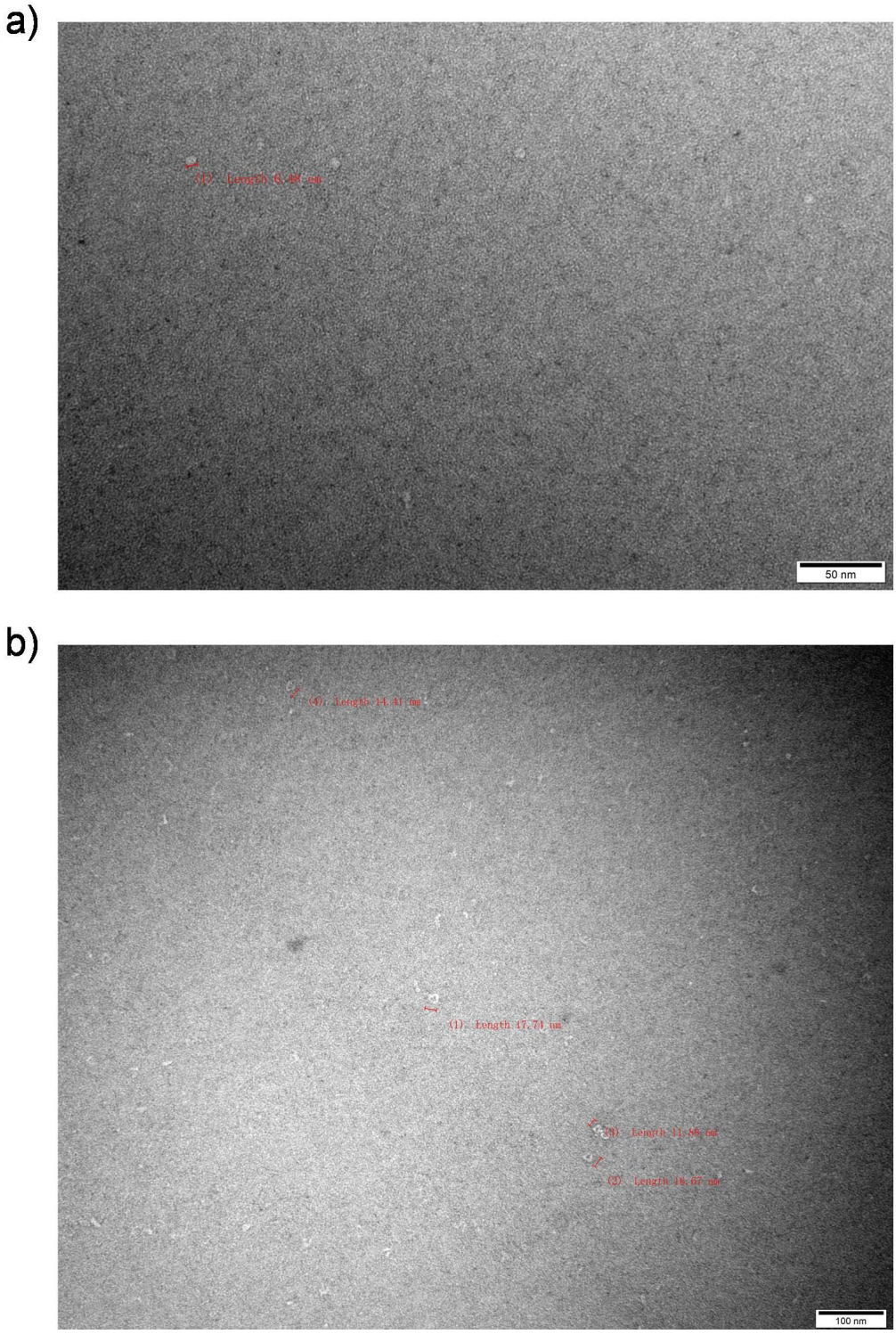


Fig. S8 Negative stain images of square, expanded DNA triangle. (a) Negative stain image of square (40D+15R). The size of square was too small to be characterized, it was only a light spot on the imaging. (b) Negative stain image of expanded triangle (93D+21D+15R).

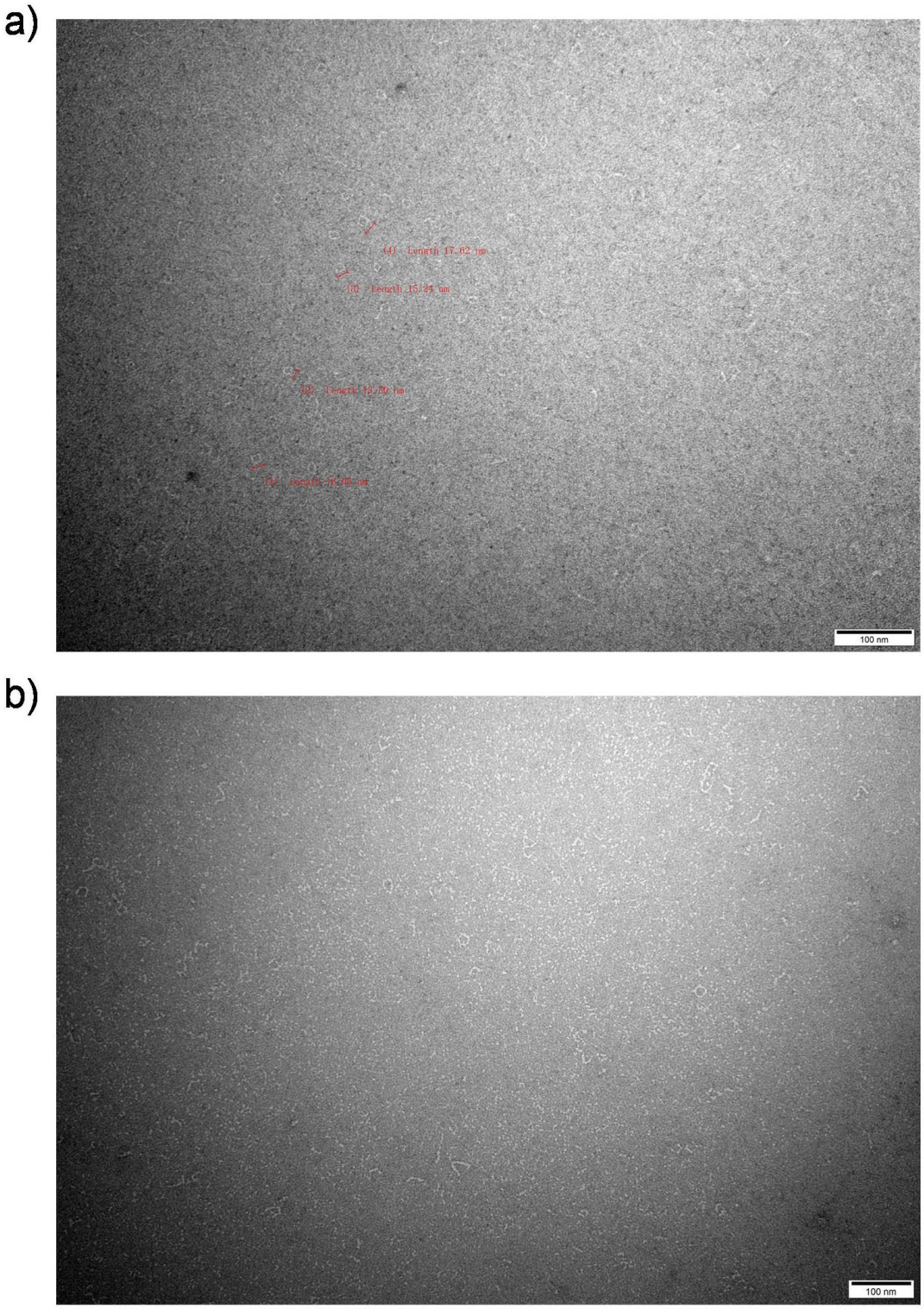


Fig. S9 Negative stain images of expanded DNA square and pentagon. (a) Negative stain image of expanded square (124D+21D+15R). (b) Negative stain image of expanded pentagon (155D+21D+15R).

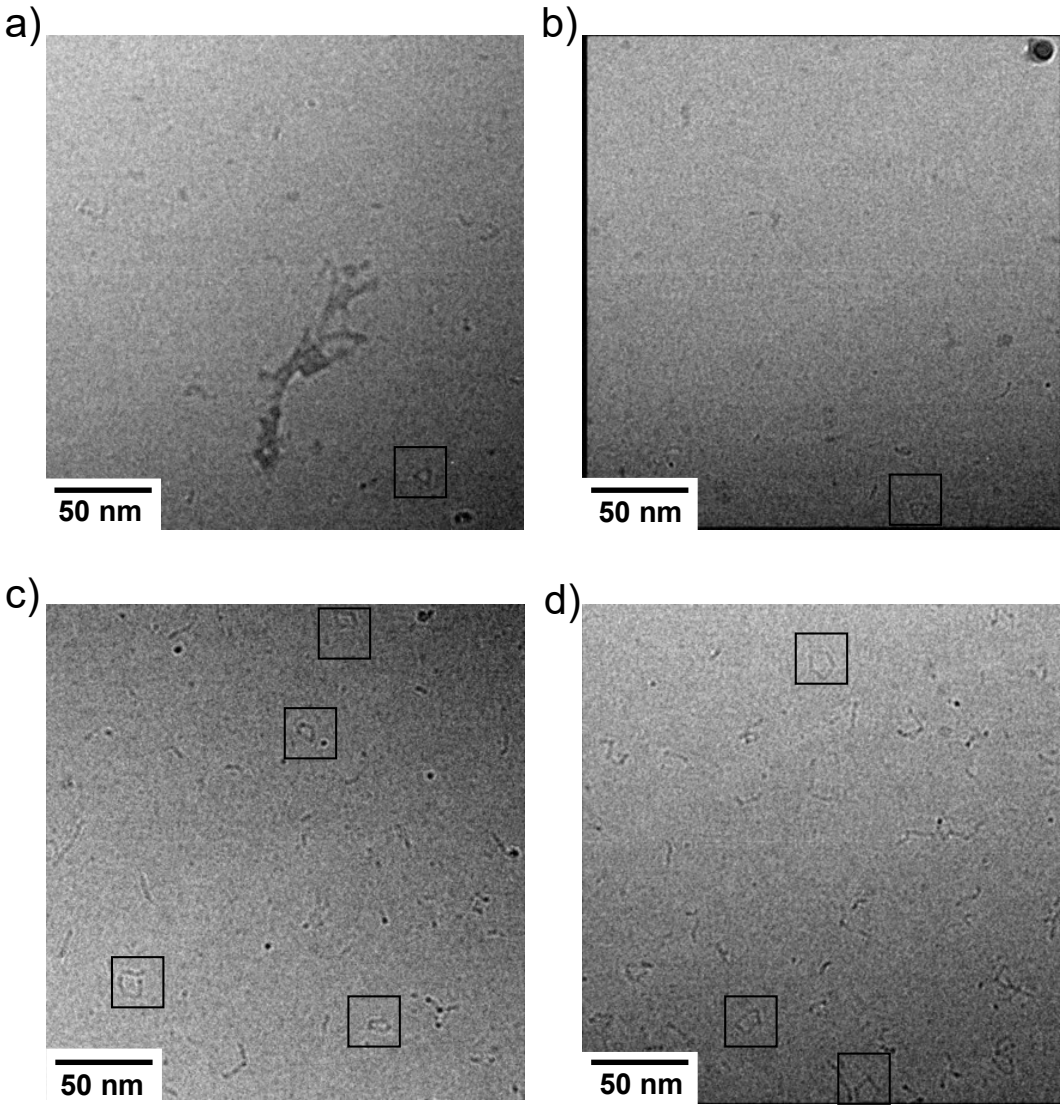


Fig. S10 Cryo-EM images of (a-b) expanded DNA triangle (93D+21D+15R), (c) square (124D+21D+15R) and (d) pentagon (155D+21D+15R).

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