Supporting Information

Single-Molecule Imaging of DNA Polymerase I (Klenow Fragment) Activity by Atomic Force Microscopy

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Reagents

The circular M13mp18 viral DNA (New England Biolabs) and Oligonucleotide staple strands (Sangon Biotech Co., Ltd., Shanghai, China) were dissolved in TAE/Mg^{2+} buffer (40 mM Tris-acetate, 1 mM EDTA, 12.5 mM $MgCl_2$, pH 8.0). The dNTPs (TaKaRa, Biotechnology Company, Dalian, China) and Cy5-dCTP (GE Healthcare) were stored at -20 °C and diluted in the provided buffer before use. Klenow fragment (KF) stock (New England Biolabs) was aliquoted and frozen at -20 °C prior to use. All water used was 18.2 MΩ·cm^{-1} Milli-Q water prepared from a Millipore system (Germany).

Experimental section

DNA replication in vitro. For AFM imaging experiments on the mica surface, excess dNTPs (dATP, dGTP, dTTP, and dCTP) and KF were incubated with DNA origami (the template DNA was fixed) in TAE/Mg^{2+} buffer at 25 °C for ~15 min. The concentrations of the reaction complex were ~0.1–0.5 mM dNTPs, 0.1–0.5 U/μL KF, and 0.5–3.0 nM origami. A 3-μL drop of the reaction complex was then placed on a freshly cleaved mica surface and incubated 3–5 min; thereafter, 30 μL TAE/Mg^{2+} buffer was added into a liquid cell. The resulting sample was imaged by AFM.

For FRET experiments, the reaction was carried out as mentioned above, but Cy5-dCTP and Cy3-A17 were used instead of dCTP and A17 to measure fluorescence emission before and after replication. The fluorescence intensity was recorded after adding the reaction complex (0.1–0.5 mM dNTPs, 0.01–0.05 U/μL KF, and 0.1–1.0 nM origami) using a fluorometer.

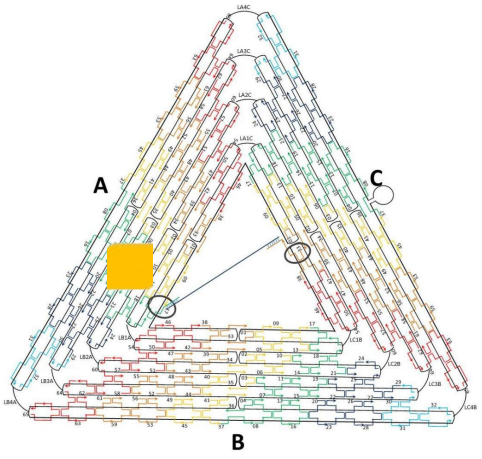

Figure S1. Schematic diagram of staple strands comprising the scaffold DNA origami and the fixing position of the DNA template (blue line between the two black circles) on the origami. The yellow square indicates the six strands of DNA that form the marker on side A of the origami.

Sequences of DNA template.

5'-GGTAGTGTAGGACTCCTACGCGAGCGGAGACTCTAGAGTGTTCCTGATGGCCGTGAATTCAAGGCGGTGGGTGCGCGTTGCTCCTCACTTCCTCGTAATACGTTCGGAGTGTTA-3'. The sequences of the two ends were partially complementary with those of staple A17 (red line) and staple C33 (green line), respectively.

Sequences of the staple strands used for building the triangle origami. All sequences of staple strands were designed as described by Rothemund (*Nature* **2006**, 440, 297) except the two staple strands, A17 and C33 listed below, which had extended sequences to produce two sticky ends for the template DNA. Part of staple strand C33 provided the primer for replication. For FRET experiments, staple strand A17 was modified with one Cy3 molecule at its 5'-end. Sequences of the staple strands, A17 and C33 were (5'-3'):

A17: 5’-CGTAGGAGTCCTACACTACCTAGCCCGGAATAGGTGAATGCCCCCTGCCTATGGTCAGTG-3’,

A17-Cy3: Cy3-CGTAGGAGTCCTACACTACCTAGCCCGGAATAGGTGAATGCCCCCTGCCTATGGTCAGTG-3’,

C33: 5’-CGCGTCTGATAGGAACGCCATCAACTTTTACATAACACTCCGAACGTATTAC-3’.

Results

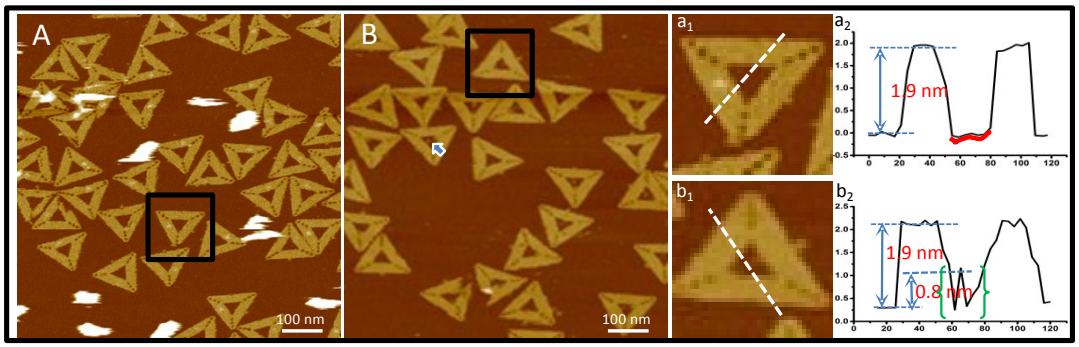

Figure S2. AFM images of triangular-shaped DNA origami on a freshly cleaved mica surface. (A) DNA origami. (a1) and (a2) show the enlarged image and height profile, respectively, from the black square in (A). The height profile revealed a flat line (red) inside the triangular origami. (B) DNA origami after hybridization with the 74-nt DNA template. The length of ssDNA was a little longer than that of the distance on the origami with the two ssDNA ends fixed; thus, the ssDNA appears as a blurred line but could be detected by the AFM probe (white arrow). (b1) and (b2) are the enlarged image and height profile, respectively, from the black square in (B). The inside of the origami presented a peak feature (green bracket), indicating the ssDNA spanning the middle of the DNA origami. The measured height of ssDNA was 0.8–1.2 nm, whereas the thickness of the origami was about 1.9–2.1 nm.

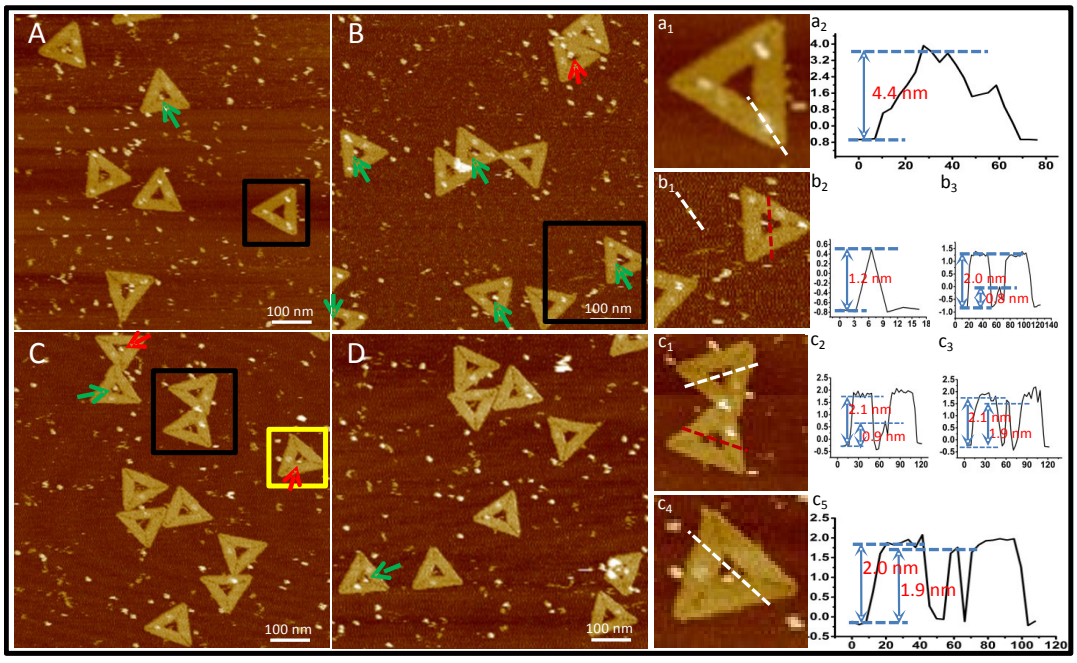

Figure S3. AFM images of the DNA replication reaction in vitro for the distribution of Klenow fragments (KFs) on the base (green arrows) and vertex (red arrows) of origami with and without dNTPs. (A and B) After KF was mixed with template DNA in a tube, the sample was placed on mica immediately (A) or after 15 min (B) for imaging. (a1), (a2), (b1), (b2) and (b3) show the enlarged images and height profiles of the black squares in (A) and (B), respectively. The measured height of a single KF molecule plus origami was 4.4 nm (a2), that of a single KF was 1.2

nm (b2), and that of the DNA template was 0.8 nm (b3). In the absence of dNTPs, the KF dots (green arrows in (A) and (B)) were mostly observed at the base of the origami. (C and D) In the presence of dNTPs, KF mostly localized to the vertex of the origami (red arrows) at the beginning of reaction (C) and after scanning for 15 min (D). (c1) shows the enlarged image from the black square in (C). (c2) and (c3) show the height profiles for (c1); the measured heights of DNA were 0.9 nm and 1.9 nm, indicating ssDNA (white line) and dsDNA (red line), respectively. (c4) and (c5) show the enlarged image and height profile, respectively, from the yellow square in (C). The measured height of the spanning DNA was 1.9 nm, indicating dsDNA. KF also preferentially localized to the angles of the origami, as shown in (a1) (white dots).

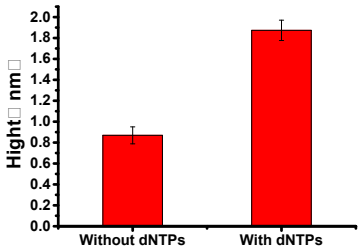

Figure S4. The measured heights of DNA on mica. Samples were imaged immediately after the template DNA, Klenow fragments (KFs), and dNTPs were mixed in tubes. Error bars indicate the standard deviation of three independent experiments from 50 DNA strands in the presence of dNTPs (with dNTPs; dots at the vertex of DNA origami) and in the absence of dNTPs (without dNTPs; dots at the base of DNA origami).

Movie S1. Time-lapse imaging of a single functional Klenow fragment molecule moving along a fixed single-stranded DNA to form a double-stranded DNA.