Electronic Supplementary Information

Nanoscopic observation of DNA crystal surface and its dynamic formation and degradation using atomic force microscopy

Haruhiko Eki,[a] Katsuhiko Abe,[a] Hiroshi Sugiyama,* [a,b] and Masayuki Endo* [a,b]

[a] Department of Chemistry, Graduate School of Science, Kyoto University, Kitashirakawa-oiwakecho, Sakyo-ku, Kyoto 606-8502, Japan.

[b] Institute for Integrated Cell-Material Sciences, Kyoto University, Yoshida-ushinomiyacho, Sakyo-ku, Kyoto 606-8501, Japan.

Experimental Procedure

Materials

The DNA sequences were used for previous research. All strands were purchased from Eurofins Genomics (Tokyo, Japan). DNA sequences used in the experiment: L: 5'-CGCACCGCGCACCGCGCACCG-3' M: 5'-GAGGAGCCTGCGCGGACAGAG-3' S: 5'-TCCTCTGTGGCTCC-3'

Assembly of DNA triangle

DNA strands L, M, and S were mixed at a molar ratio of 1:3:3 in the buffer consisted of 40 mM Tris-HCl (pH 8.0), 20 mM acetic acid, 2 mM EDTA and 12.5 mM Mg $(OAc)_2$ (TAE/Mg^{2+}). DNA concentration was 0.5 μg/μL in total. This solution was annealed from 95 °C to 4 °C in 2 h.

Native PAGE

Native gel electrophoresis was performed using a 16 % polyacrylamide (19:1 acrylamide/bisacrylamide) with 10 mM $MgCl_2$. The running buffer consisted of 1×TBE and 10 mM $MgCl_2$. Gel was run at 4 °C for 120 min (120 V). After the electrophoresis, the gel was stained with SYBR Gold.

Assembly of DNA crystal by hanging drop vaper diffusion method

Assembled DNA triangle solution (4 μL) was mixed with 5 μL buffer consisted of 50 mM Tris-HCl pH 7.6, 10 mM $MgCl_2$ and 1.6 M $(NH_4)_2SO_4$ (crystallization buffer). The drop was incubated against 0.5 mL 1.2~1.4 M $(NH_4)_2SO_4$ aqueous solution at 22 °C for several days.

Assembly of DNA crystal by sitting drop vaper diffusion method

Assembled DNA triangle solution (12 μL) was mixed with 15 μL crystallization buffer on the Micro-Bridge. The drop was incubated against 0.5 mL 1.2~1.4 M $(NH_4)_2SO_4$ aqueous solution at 22 °C for several days.

AFM imaging

A crystal was transferred as a 5 μL drop onto a freshly cleaved mica surface using a Cryo-loop. For AFM imaging, the drop was consisted of the mixture of 0.5 μg/μL DNA triangle and crystallization buffer at a volume ratio of 4:5. For observation of the assembly, the drop consisted of the mixture of 1.0 μg/μL DNA triangle and crystallization buffer at a volume ratio of 4:5 was used. AFM images were obtained using Dimension FastScan AFM (Bruker AXS, Madison, WI) with a silicon nitride cantilever with a spring constant of 0.06-0.14 N/m and resonant frequency of 98.5-140 kHz in water. Scanning was performed in the buffer solution using a tapping mode.

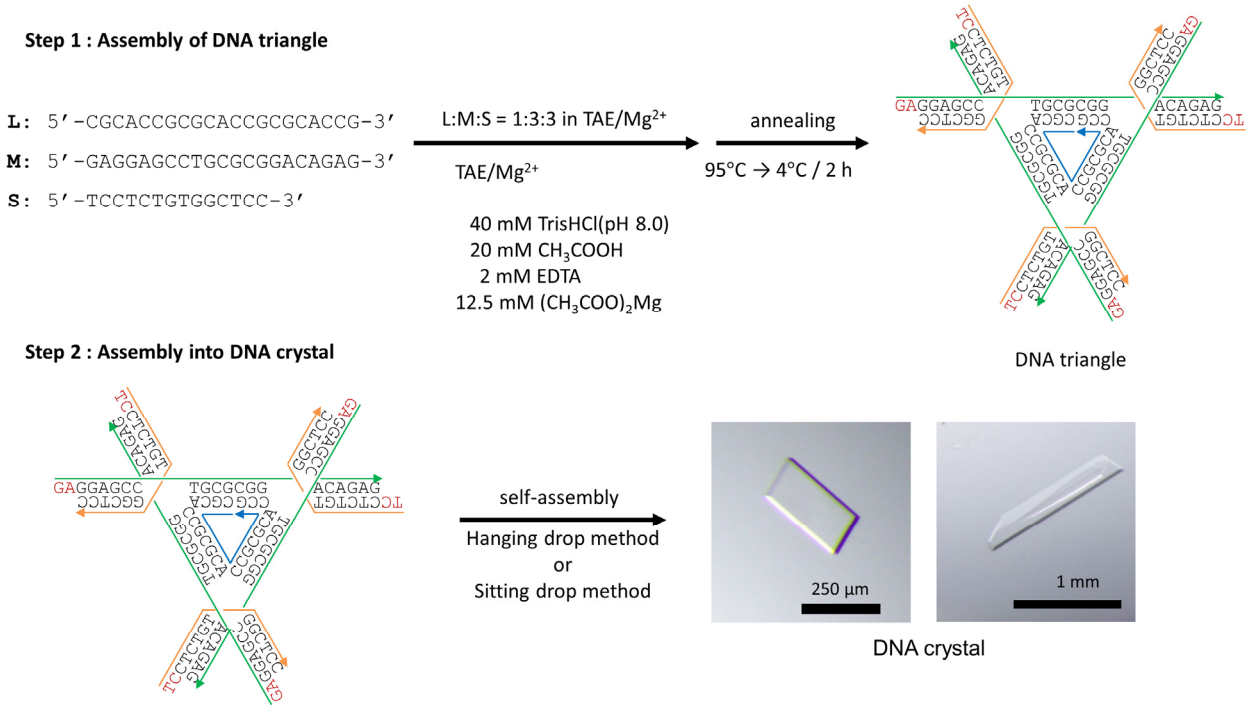

Figure S1. Preparation of DNA tensegrity triangle and crystallization. The sequences for DNA triangle are presented as L, M, and S strand. [1] DNA crystals prepared using hanging drop method (left image) and sitting drop method (right image).

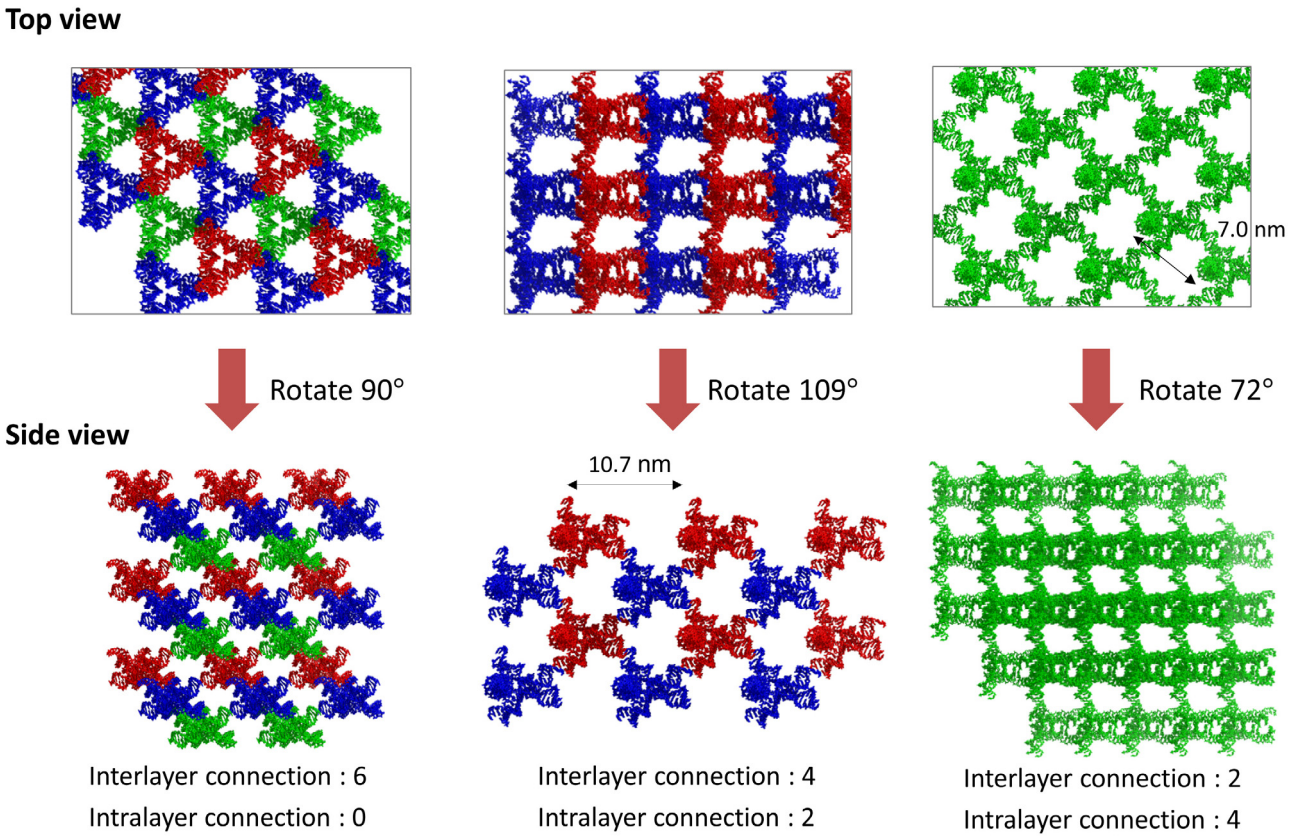

Figure S2. Possible surface lattice structures viewed from top and side. We categorized these by using the numbers of interlayer and intralayer connections.

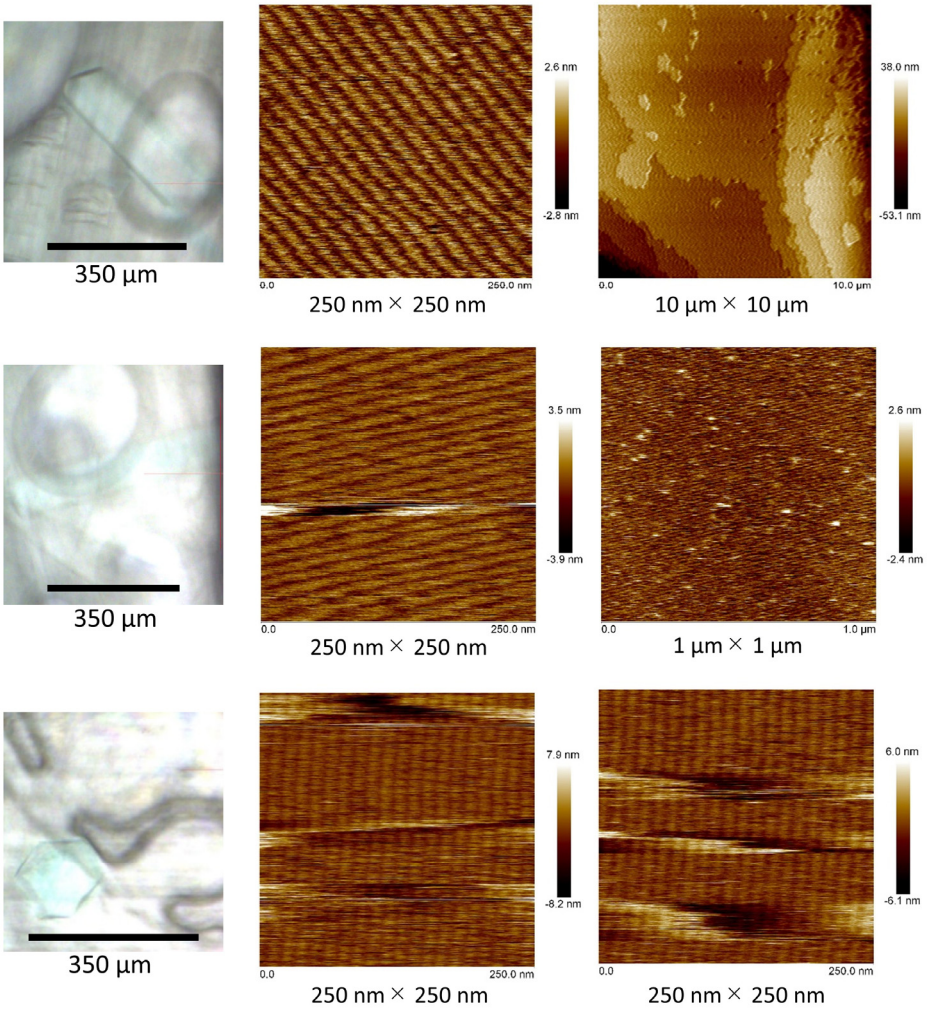

Figure S3. Microscope images of DNA crystals and AFM images of their DNA crystal surface. According to the surface pattern, the surface lattice structure of these crystals can be categorized to the pattern in Figure S2 middle.

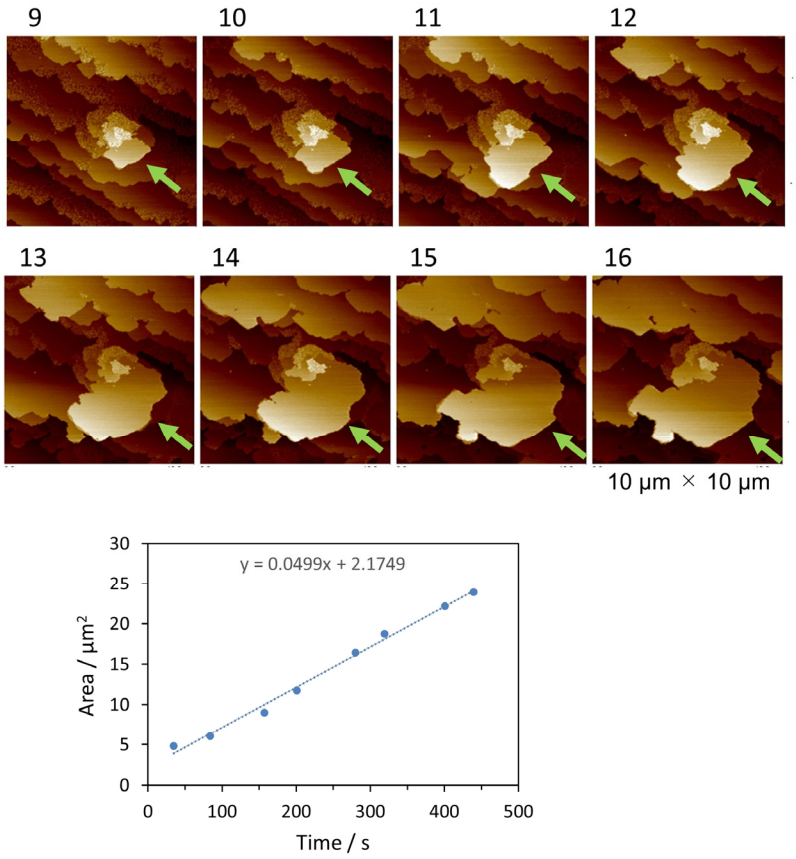

Figure S4. Measurement of planar dimension of terrace in Figure 3c. Images were obtained at 1 frame/min. Planar dimension of terrace (arrow) was measured using ImageJ and plotted. The planar dimension increased linearly.

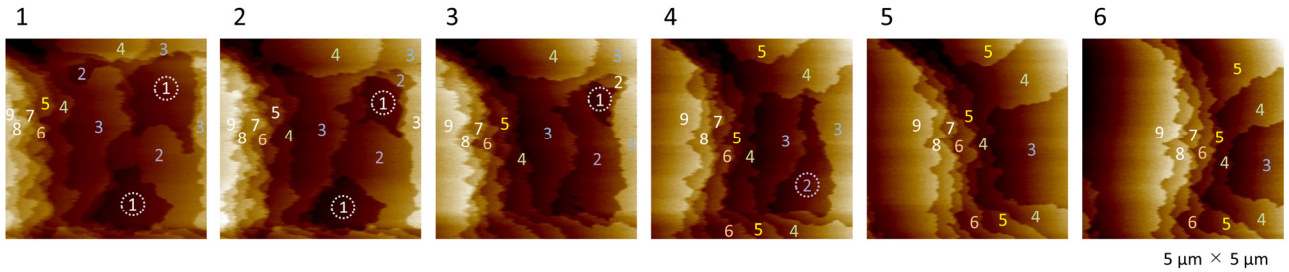

Figure S5. Time-lapsed AFM images of the crystal growth by AFM. AFM observation of growth of the layers and filling the cavities (circled number). The number in the images presents a layer of a terrace. Images were obtained at 1 frame/min.

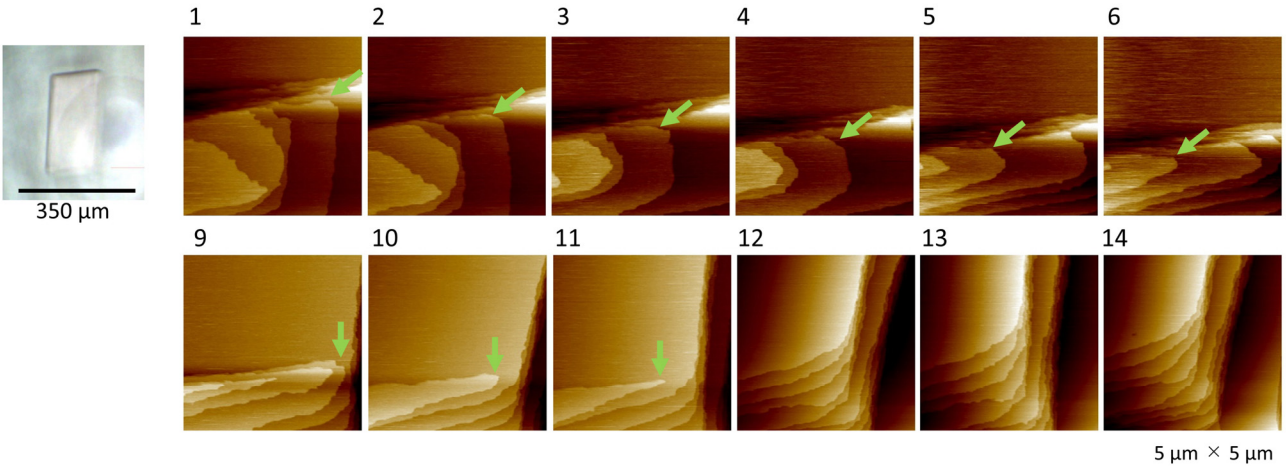

Figure S6. Time-lapsed AFM images of degradation of the crystal. Arrows represent the edges of the terrace. Images were obtained at 1 frame/min.

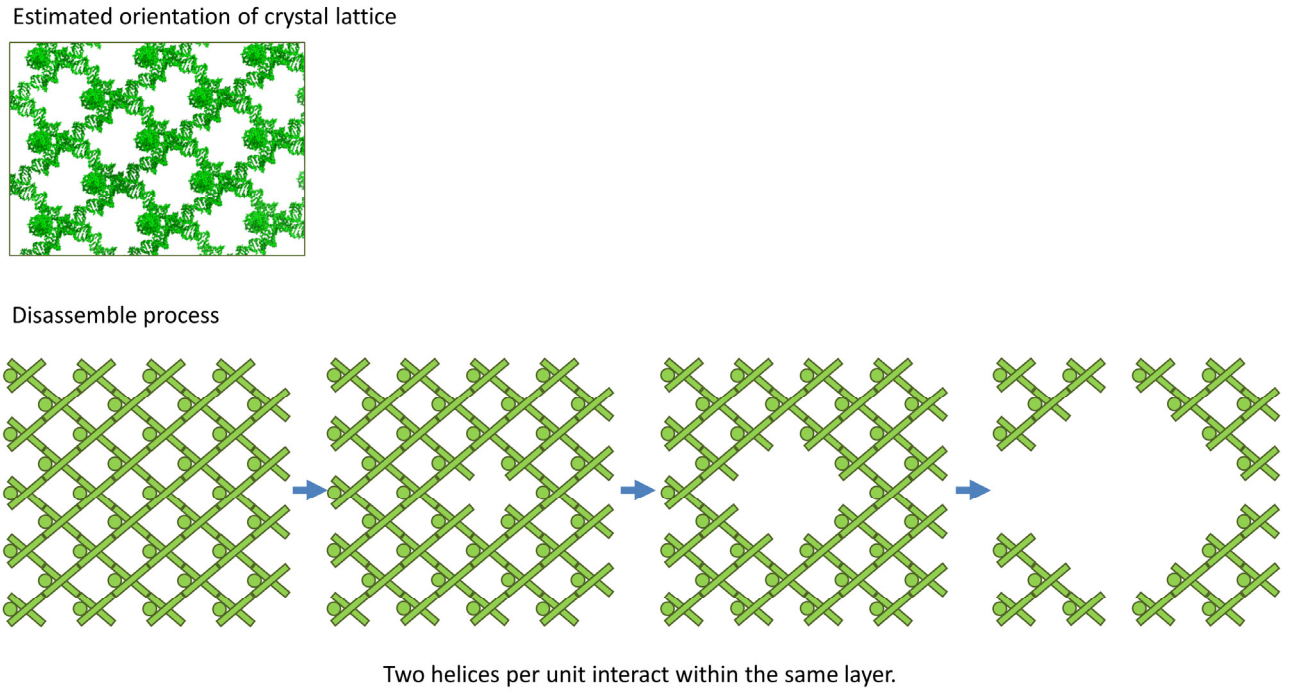

Figure S7. Estimated degradation mechanism of the crystal. Ordered disassembly of the triangle units.

Movie S1. Time-lapsed AFM movie of crystal growth in Figure 3b. Imaging was performed in a buffer condition described in experimental procedure. AFM images were recorded at 1 frame/min. Image size: 3 μm × 3 μm.

Movie S2. Time-lapsed AFM movie of screw dislocation crystal growth in Figure 3c. Imaging was performed in a buffer condition described in experimental procedure. AFM images were recorded at 1 frame/min. Image size: 10 μm × 10 μm.

Movie S3. Time-lapsed AFM movie of crystal degradation in Figure 4. Imaging was performed in a buffer condition described in experimental procedure. AFM images were recorded at 1 frame/min. Image size: 5 μm × 5 μm.

Reference 1. Zhao, J. et al. Post-Assembly Stabilization of Rationally Designed DNA Crystals. *Angew. Chem. Int. Ed.* **54**, 9936-9939 (2015).