Electronic Supplementary Information

Supramolecular 1-D polymerization of DNA origami through a dynamic process at the 2-dimensionally confined air-water interface

Yusuke Yonamine,*[a,b,c] Keitel Cervantes-Salguero,[d] Kosuke Minami,[a] Ibuki Kawamata,[d] Waka Nakanishi,[a] Jonathan P. Hill,[a,b] Satoshi Murata,*[d] and Katsuhiko Ariga*[a,b]

[a]*World Premier International (WPI) Research Centre for Materials Nanoarchitectonics (MANA), National Institute for Materials Science (NIMS) 1-1 Namiki, Tsukuba, Ibaraki, 305-0044, Japan*
[b]*CREST, JST, Sanbancho, Chiyoda-ku, Tokyo, 102-0075, Japan*
[c]*Department of Chemical Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan*
[d]*Department of Bioengineering and Robotics, Tohoku University, 6-6-1 Aobayama, Sendai 980-8579, Japan*

Contents

1. Preparation of a DNA sheet.

The reported DNA sheet having a flexible hinge was assembled in a similar manner using the same sequences.[1] M13 mp18 Single Strand DNA (final concentration: 5 nM; Takara Bio Inc.) and staple DNAs (final concentration: 50 nM each; Eurofins Genomics) without any cholesterol groups were dissolved in Tris-acetate-EDTA containing magnesium ion buffer (Tris 40 mM, acetate 20 mM, EDTA 1 mM and magnesium acetate 12.5 mM; TAE/Mg^{2+}) and annealed at 90 °C. The solution was cooled to 20 °C at 0.13 °C per minute. After the incubation, the sample solution (500 μL) was centrifuged in a 100 kDa Amicon centrifugal filter (Millipore) at 10,000 g for 2 minutes to remove the extra staple DNAs. TAE/Mg^{2+} (450 μL) was added to the residual sample solution (50μl) and the filtration step was repeated three times. The DNA sheet solution was diluted to 5 nM with TAE/Mg^{2+}.

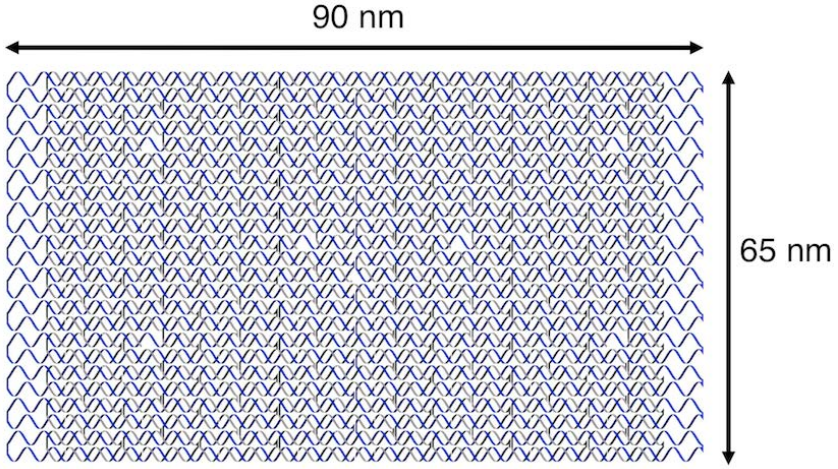


Fig. S1. Schematic layout of a DNA origami sheet having single strand DNA loops (32 bases each) on the shorter edges.

2. Preparation of lipid-modified DNA sheets.

Lipid-modified DNA sheets were prepared by a previously reported method.[2] An aqueous solution of dioctadecyldimethylammonium bromide $2C_{18}N^+$ (8 μL, 3.3 mM; 1 mol equivalent to phosphate groups of DNA) was added to 400 μL of DNA sheet solution (5 nM) in TAE/Mg^{2+} buffer at 0 °C. A water-insoluble white precipitation formed immediately. The dispersion was centrifuged at 12,000 g for 5 minutes and the supernatant was removed. The resultant precipitation was dissolved in 400 μL of chloroform solution.

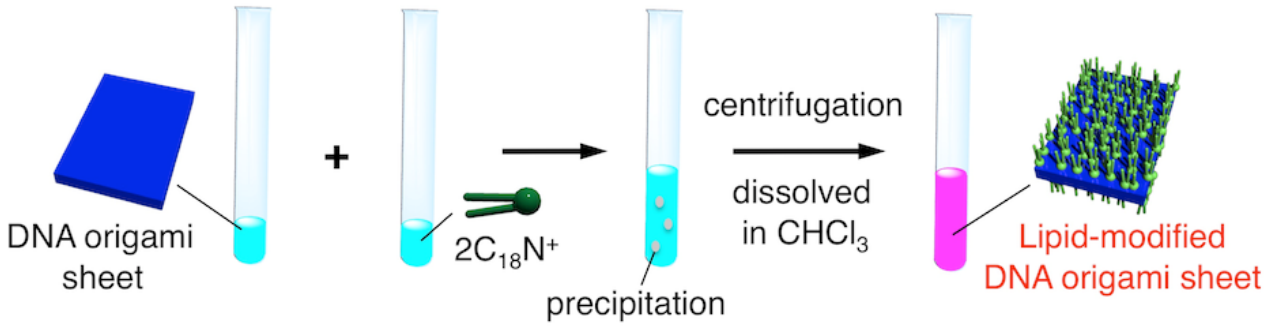


Fig. S2. Schematic diagram of the procedure for preparation of lipid-modified DNA origami sheet.

3. Characterization of lipid-modified DNA sheets.

UV-Vis absorbance of a chloroform solution of DNA sheets at 260 nm was measured (UV-3600, SHIMADZU) as being 0.263 (Fig. S3a, red line) while the absorbance of the aqueous supernatant was significantly decreased to 0.021 (Fig. S3a, blue line) compared to the value before mixing (0.392; Fig. S3a, black line). The yield of extraction was calculated to be 67% by assuming that the molar extinction coefficient of DNA remains constant. Lipid-modified DNA sheets in chloroform solution were analyzed by using circular dichroism (CD) spectroscopy (J-820, JASCO). The spectrum showed a Cotton effect pattern closer to the B-type (Fig. S3b, red line) similar to the standard DNA sheet in TAE/Mg^{2+} buffer (Fig. S3b, black line). Fourier transform-infrared (FT-IR) spectra of the cast films were obtained using an FT-IR spectrometer (Nicolet NEXUS 670 FT-IR). Sample solutions were cast onto a BaF_2 window and spectra were recorded in transmission mode. Data were collected at a spectral resolution of 4 cm^{-1}. In the case of DNA samples, DNA sodium salt from calf thymus (Nacalai tesque, Inc.) in pure water was used as a reference material of the double helical structure to avoid peaks due to the buffer components in the DNA origami sheet sample.

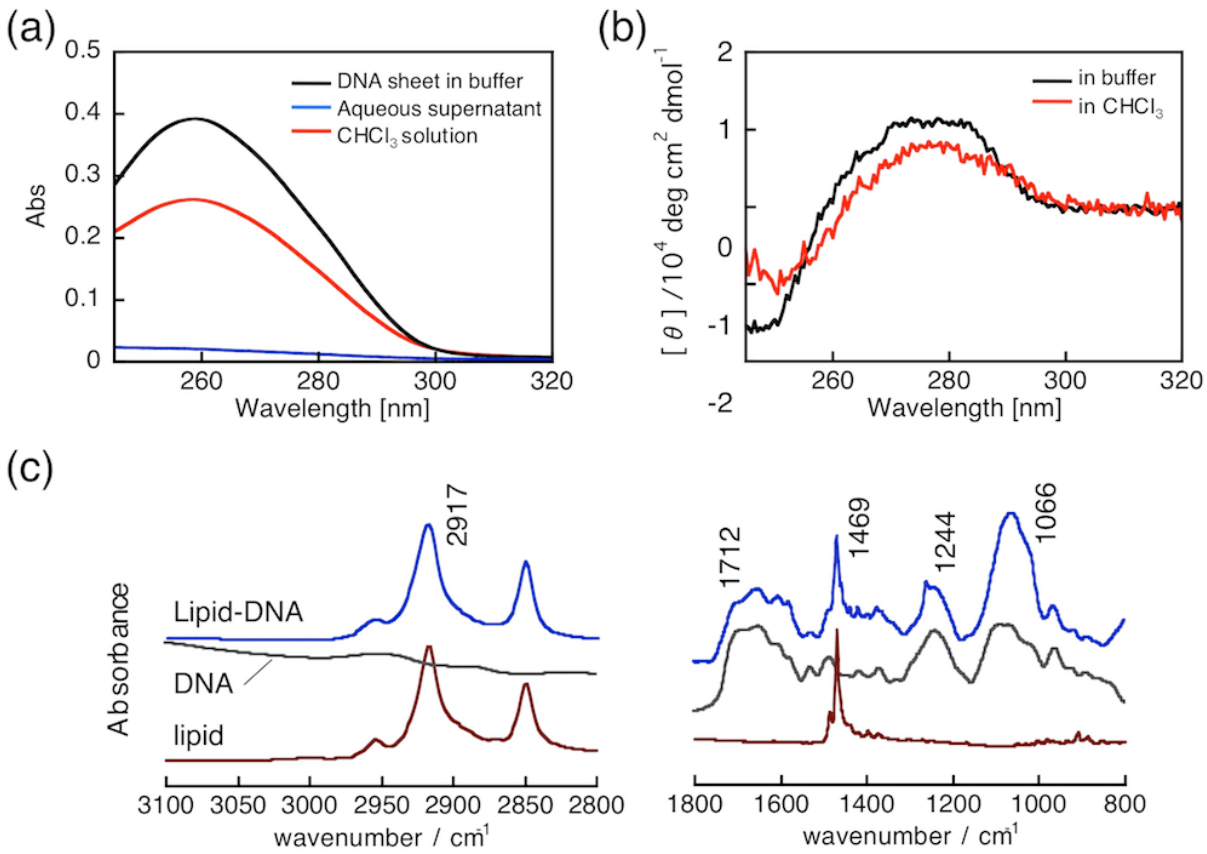


Fig. S3. (a) UV-Vis spectra of chloroform solution (red line), aqueous supernatant (blue line), and buffer solution of DNA sheets before mixing (black line). (b) CD spectra of chloroform solution (red line) and buffer solution (black line) of DNA origami sheets. Baseline: $CHCl_3$ (red line) and TAE/Mg buffer (black and blue lines), respectively. (c) FT-IR spectra of cast films of lipid-modified DNA sheets (blue), double-stranded DNA from calf thymus (gray) and $2C_{18}N^+$ lipid (brown).

4. Preparation of a Langmuir–Blodgett film of lipid-modified DNA sheets.

The measurement of surface pressure–area (p–*A*) isotherms and Langmuir–Blodgett (LB) film preparation were performed on a USI-3-777C3 LB system (USI Co., Fukuoka). The trough contains 250 mL pure water subphase and has a working area of 334 cm^2. The lipid-modified DNA sheets or $2C_{18}N^+$ lipid in chloroform solution was spread on the pure water. After 15 min had lapsed (for evaporation of the chloroform), the film was compressed at a rate of 0.2 cm^2 sec^{-1} to the predetermined surface pressures at 20.0 ± 0.2 °C unless otherwise noted. Films at the air–water interface were transferred onto freshly cleaved mica substrates at a pressure of 32 mN/m by lifting the pre-immersed substrates vertically at a rate of 0.02 mm sec^{-1}. AFM imaging was performed using an AFM system (Nanoscope V, Bruker) with a cantilever operating in dynamic force mode (SI-DF40, Hitachi High-Tech Science) under dry conditions.

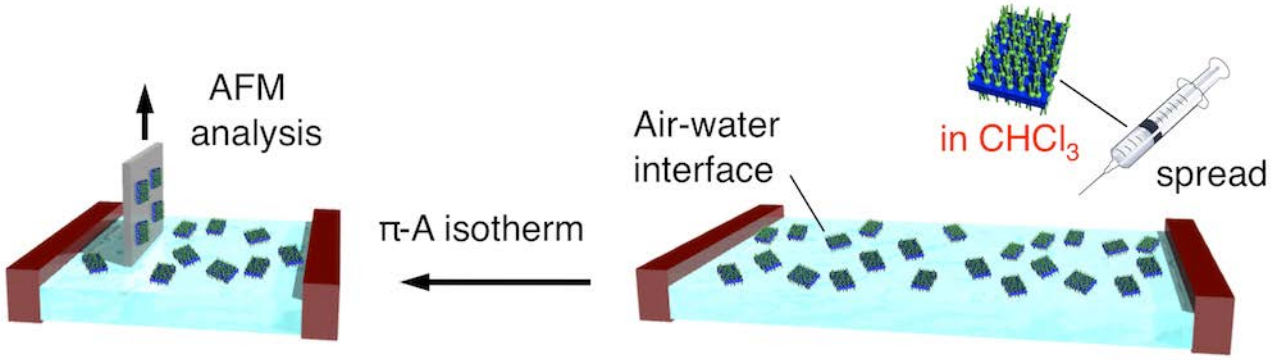


Fig. S4. Schematic diagrams of the procedure for a Langmuir-Blodgett film of a lipid-modified DNA sheet.

5. AFM images of LB films of DNA origami sheets under various conditions.

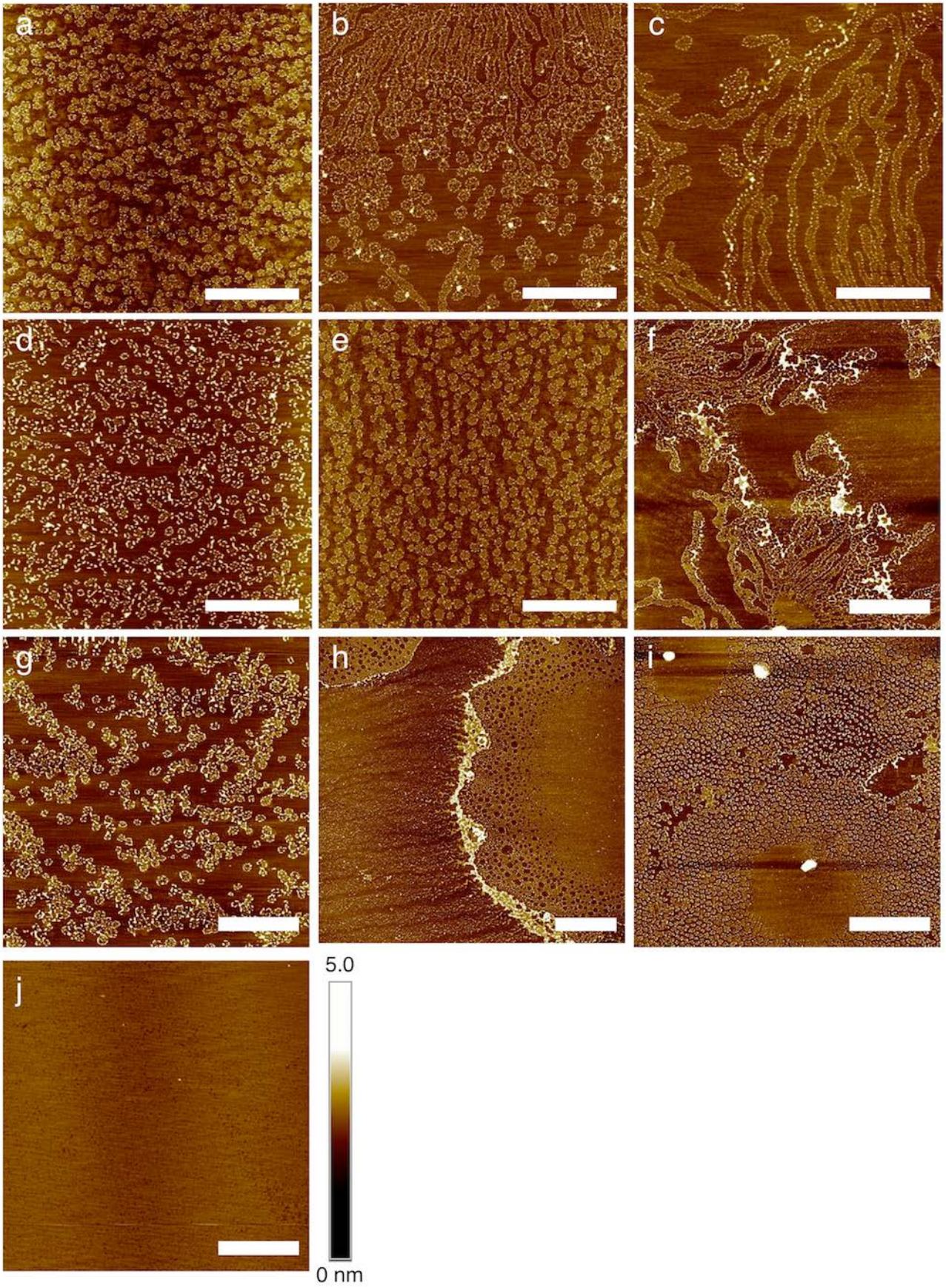


Fig. S5. AFM images of LB films of DNA origami sheets under various conditions. (a)-(j) correspond to the entries in Table 1 in the main article. Scale bars: 1 μm.

6. Analyses of AFM images for DNA sheet polymer lengths.

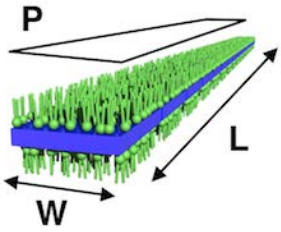
The lengths of DNA sheet polymers were analyzed from the AFM images of the LB films (Fig. S6a–e) using Image J software.[3,4] The contrast of an image was enhanced and prepared as a binary image. Noise was removed and the perimeters of DNA sheet polymers were measured using the "analyze particles" tool. In the case of fused DNA sheet polymers, adhesion points were cut manually. Large two-dimensional aggregates were not processed in the analyses. The resultant images are shown in Fig. S6f–j.

Length measurement

The length of the DNA sheet polymer L was calculated by equation S1;

$$L = \frac{P - 2W}{2} \tag{S1}$$

where P and W are the perimeter of the DNA sheet polymers and theoretical width of DNA sheet (70 nm), respectively.



Population of isolated DNA sheets

DNA sheet structures of lengths less than 200 nm in Fig. S6f–j were counted as isolated DNA sheet monomers. The number was divided by area (25 μm^2) to obtain the population density.

Length–weighted average length

Length-weighted average length (L_l) and number average length (L_n) of DNA sheet polymers are defined as below:

$$L_l = \frac{\sum_i L_i N_i \times L_i}{\sum_i L_i N_i} \quad (i = 1, 2, 3 \cdots) \tag{S2}$$

$$L_n = \frac{\sum_i N_i \times L_i}{\sum_i N_i}$$

where L_i is the length of each DNA sheet polymer and N_i is the number of a particular length of DNA sheet polymers

Polydispersity index

Polydispersity indices (PDI) based on DNA polymer lengths were calculated as below (see Fig. S6k).

$$\mathrm{PDI} = L_l / L_n \tag{S3}$$

Relative length distributions

Relative length distributions (%) were calculated as the ratio of a monomer existing in a specific length polymer (Fig. S6k). The value of the monomer length was used as 100 nm.

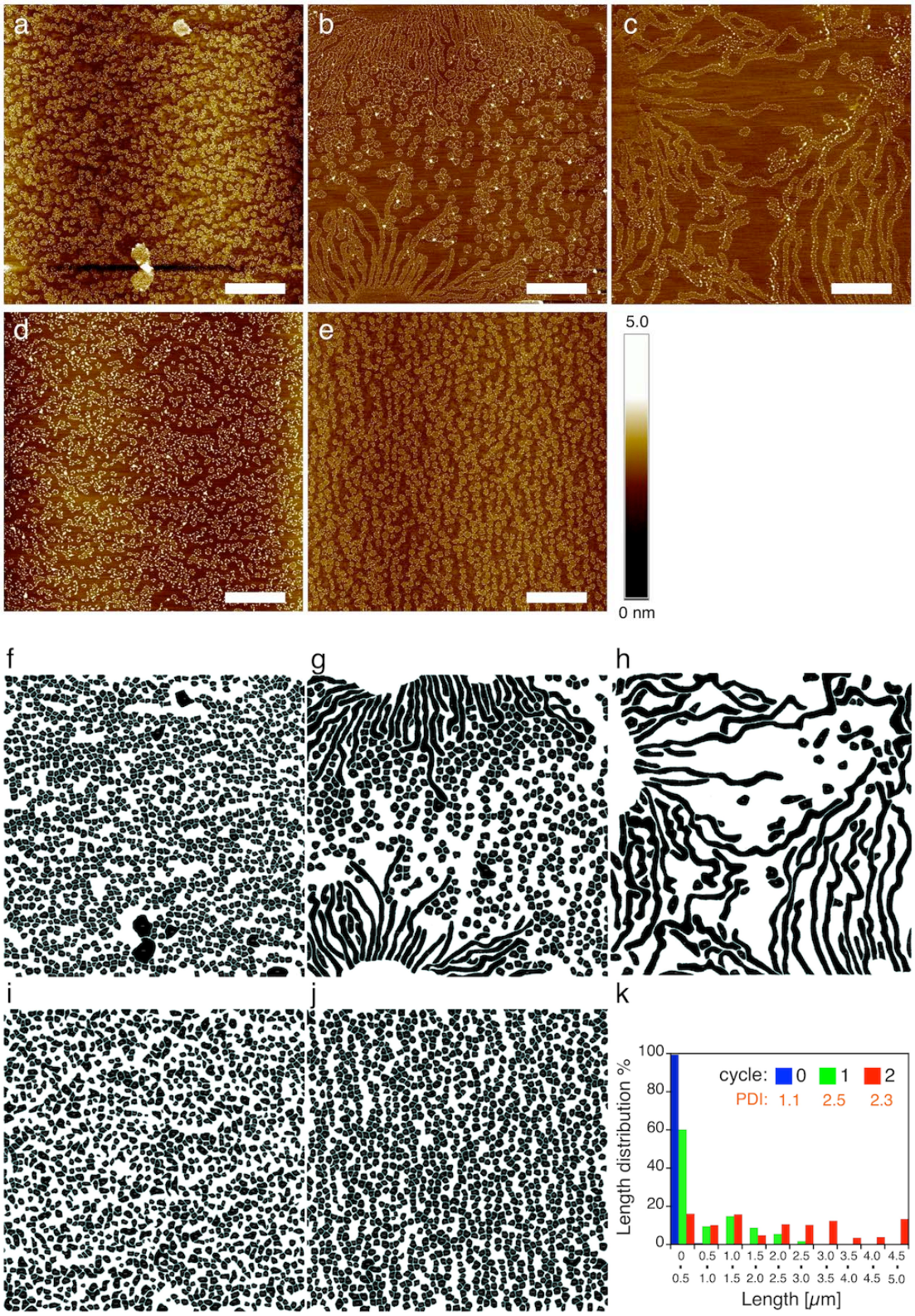


Fig. S6. AFM images and their analyses of Langmuir films of DNA origami sheets. (a–c) AFM images after compression (30 mN/m) and expansion (3 mN/m) cycles; 0 (a), 1 (b) and 2 cycles (c), respectively. (d,e) AFM images after stationary compression at 3 mN/m (d) or 30 mN/m (e) for 30 min. (f–j) Particle size analysis of DNA sheet structures. The data were extracted based on the AFM images of a–e, respectively. (k) Relative length distributions (%) of fused lipid-modified DNA origami sheets; blue: 0, green: 1, and red: 2 cycles, respectively. Polydispersity indices (PDI) were increased after the cycles due to the varied distribution of the DNA polymer lengths. Scale bars: 1 μm.

7. AFM images of DNA sheet structures after compression-expansion processes.

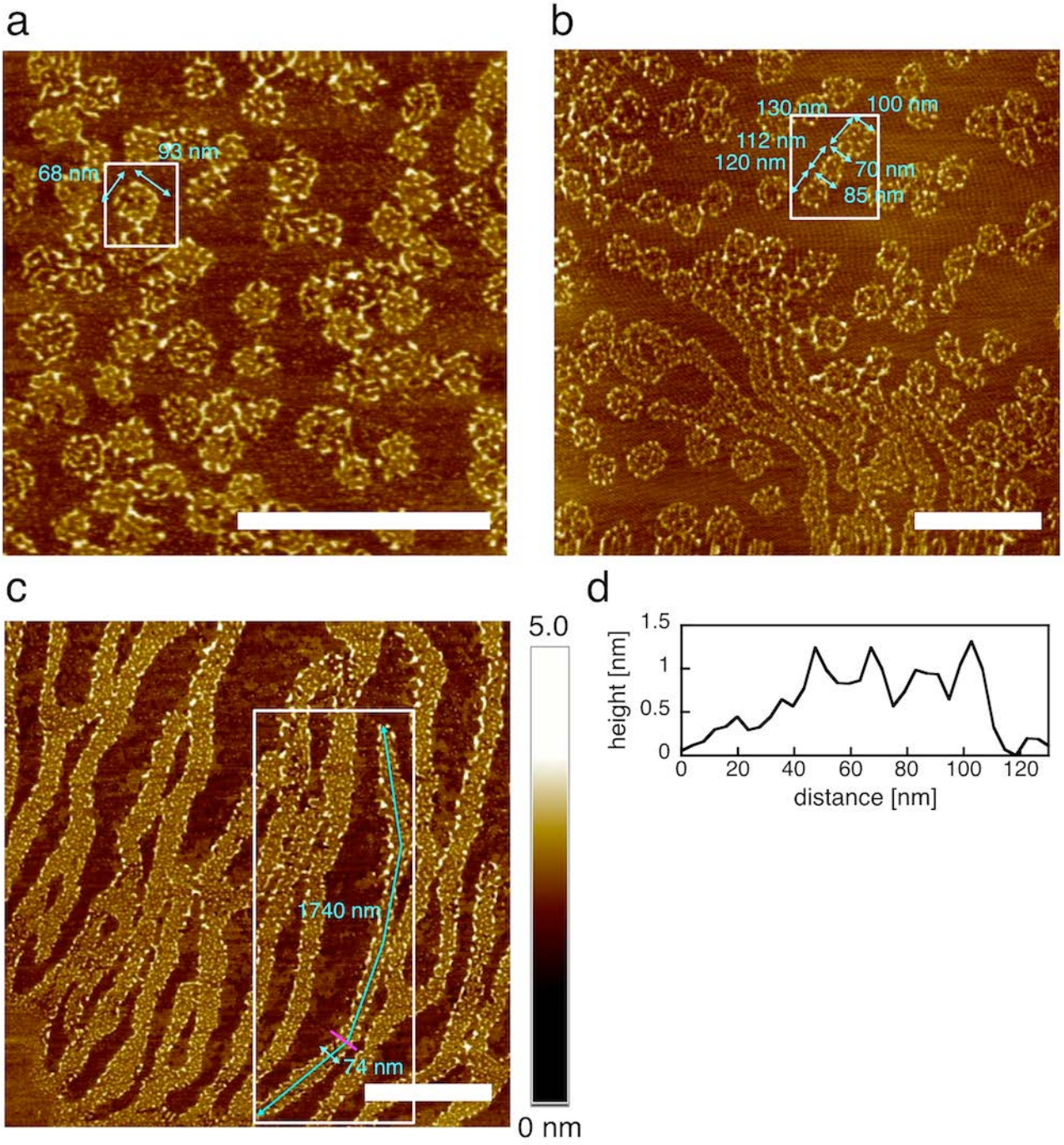


Fig. S7. (a–c) AFM images of DNA sheet structures after 0 (a), 1 (b) or 2 (c) cycles of a compression-expansion process. Scale bars: 500 nm. The regions surrounded with white boxes were shown in Fig. 4a–c. The lengths were measured using Image J software.[1,2] (d) The height profile of 1-D belt DNA structure at the magenta line in c.

8. FT-IR reflection absorption spectrometry (RAS) analyses of lipid-modified DNA sheets.

Langmuir films of lipid-modified DNA sheets (9 layers) were transferred from the air–water interface onto gold-coated glass substrates at a pressure of 32 mN/m. Fourier transfer-infrared (FT-IR) spectra of the LB films on gold-coated glass (9 layers) were obtained using an FT-IR spectrometer (Nicolet NEXUS 670FT-IR) equipped with an MCT detector (for reflection absorption spectroscopy; RAS). All data were collected by the RAS method at a spectral resolution of 4 cm^{-1}.

9. References.

(1) J. List, M. Weber and F. C. Simmel, *Angew. Chem. Int. Ed.* 2014, **53,** 4236.

(2) Y. Yonamine, K. Cervantes-Salguero, W. Nakanishi, I. Kawamata, K. Minami, H. Komatsu, S. Murata, J. P. Hill and K. Ariga, *Phys. Chem. Chem. Phys.* 2015, **17,** 32122.

(3) W. S. Rasband, ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2012.

(4) C. A. Schneider, W. S. Rasband and K. W. Eliceiri, *Nat. Methods* 2012**, 9,** 671.