Supporting Information

Measurement of Nanomechanical Properties of DNA Molecules by PeakForce Atomic Force Microscopy based on DNA Origamis

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Experimental section

Preparation of bilayer DNA origami

The designed cross-shaped DNA origami and triangular DNA origami were prepared in 1× TAE-Mg^{2+} buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate, pH 8.0) as previously reported by references 1 and 2, respectively.[1,2] Briefly, the DNA origami self-assembly process was carried out by mixing 2 nM single-stranded M13mp18 DNA (New England Biolabs, Ipswich, MA, USA) with a 10-fold molar excess of staple stands (Takara Bio, Shiga, Japan). The mixture was annealed from 95 ℃ to 4 ℃ at a speed of 0.1 ℃/10 s. The excess staple strands were removed by ultrafiltration three times with 1× TAE-Mg^{2+} buffer using 100-kDa cut-off filters (Amicon, Millipore, Billerica, MA, USA).

Immobilization of bilayer DNA origami on mica surface

The prepared DNA origami sample in TAE buffer was deposited on the surface of freshly cleaved mica and incubated for 2 min. A high concentration of triangular DNA origami was used to form bilayer triangular DNA origamis on the mica surface.

Immobilization of the DNA origami and single lambda DNA molecule on the mica surface

The freshly cleaved mica surface was first modified with 100 nM Ni^{2+} for 3 min. After excess Ni^{2+} was washed off three times with water, the sample containing both types of DNA was deposited onto the modified mica surface.

AFM measurement using PeakForce Quantitative Nano-Mechanics (PF-QNM)

AFM in PF-QNM mode was performed in 1× TAE/Mg^{2+} buffer at different loading peak forces (60–140 pN) after the sample was mounted on AFM (Multimode IIIV, Bruker, Santa Barbara, CA, USA). A probe (Scanasyst-Fluid^{+}, Bruker) with a nominal spring constant of 0.7 N/m was used unless otherwise stated. To obtain a more precise Young's modulus (*E*) value, the deflection sensitivity of the probe was calibrated on the mica surface several times and the mean value was taken. The spring constant of the probe was determined using the Thermal Tune function of the AFM software, and the radius (R) of the tip was calibrated using a standard sample of known Young's modulus (Bruker). After calibration, tip deflection sensitivity was determined to be ~22.7 nm/v, k was ~0.7 N/m, and the tip radius was ~2 nm. Imaging

scanning frequency was fixed at 1.0 Hz. Image and data processing were performed using commercial NanoScope Analysis Software version 6.3 (Bruker).

Determination of the *E* value of DNA origami on the mica surface

DNA origami was topographically and mechanically imaged in PF-QNM mode. The *E* value was determined by fitting the data to the Derjaguin-Muller-Toporov (DMT) model, which is used for samples with relatively weak adhesion forces and using a tip with a small radius. The DMT model was calculated as follows:

$$F - F_{adh} = \frac{4}{3}E^*\sqrt{R(d-d_0)^3} \quad (1)$$

Where F-F_{adh} is the loading force and adhesion force, E^* is the E value, R is the contact radius, and d-d_0 is the indentation depth. E^* was obtained using the following formula:

$$E^* = \left(\frac{1-V_S^2}{E_S} + \frac{1-V_t^2}{E_t}\right)^{-1} \quad (2)$$

Where E_s and E_t are the radial compression elastic moduli of the sample and needle tip, respectively; and V_s and V_t are the Poisson ratios of the sample and needle tip, respectively. When analyzing biomolecules,[3] the hardness of the tip has a much greater effect than that of the samples; therefore, the last term can be negligible. A Poisson's ratio value of 0.3 was used for *Vs* because we treated DNA as incompressible.
The *E* value was calculated from the slope of Equation 1.

Bottom effect elastic corrections of the *E* value of DNA origami on the mica surface[4]

In the case of $\delta \leqslant R$, the force can be expressed as

$$F = F_0\left[\frac{1}{h^0} + \frac{1.133\sqrt{\delta R}}{h} + \frac{1.497\delta R}{h^2} + \frac{1.469\delta R\sqrt{\delta R}}{h^3} + \frac{0.755(\delta^2 R^2)}{h^4}\right] \quad (3)$$

$$F_0 = \frac{16}{9}E_{\text{DNA Origami}}\sqrt{R}\delta^{3/2} \quad (4)$$

Where F is the force on the DNA Origami, h is the sample thickness , δ is the indentation depth ,R is the probe radius, $E_{\text{DNA Origami}}$ is Young's modulus of DNA Origami. For the DNA origami, the Poisson coefficient should be around 0.3, then, the factor 16/9 that appears in (4) should be replaced by 1.49. Therefore, (4) is replaced by

$$F_0 = 1.49\ E_{\text{DNA Origami}}\sqrt{R}\delta^{3/2} \quad (5)$$

δ was obtained by analyzing the FD curve using the image in PF-QNM mode, and represents the distance from the contact start point to the place where the corresponding force was applied.

Supporting Figures

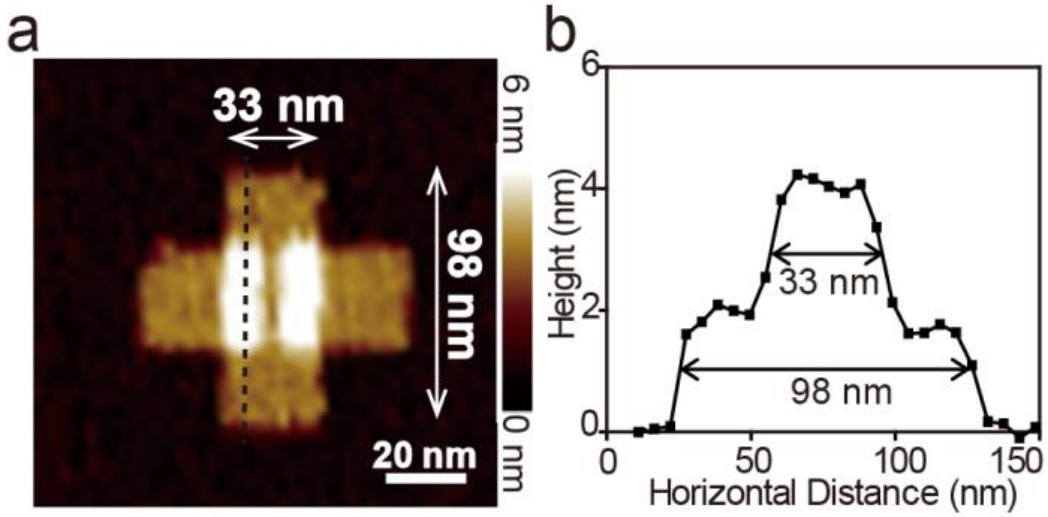

Figure S1. AFM PF-QNM images of the shape and dimensions of the cross-shaped DNA origami adsorbed onto the mica surface. (a) Topographic image. (b) Height profile (right) along the black line in (a).

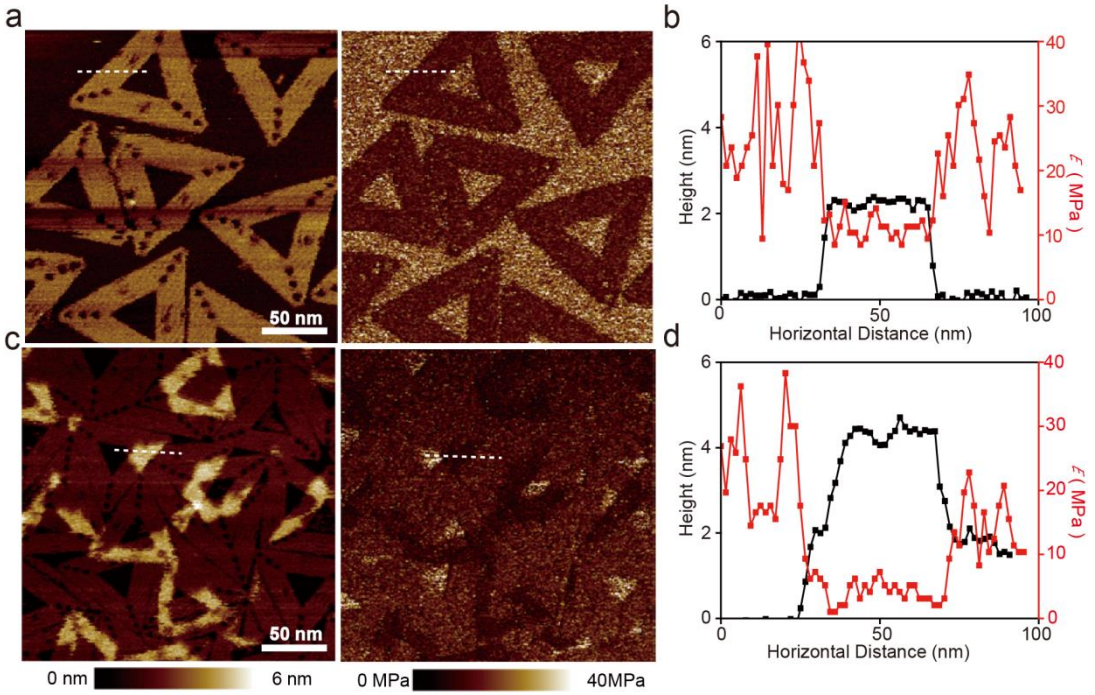

Figure S2. AFM PF-QNM images characterizing the mono- and bilayer triangular DNA origamis. (a) Height (nm) (left) and stiffness (MPa) (right) of the monolayer triangular DNA origamis. (b) Cross-sectional profiles of the height and DMT of monolayer DNA origamis. (c) Height (nm) (left) and stiffness (MPa) (right) of the bilayer triangular DNA origamis. (d) Cross-section profiles of height and *E* for the bilayer DNA origamis.

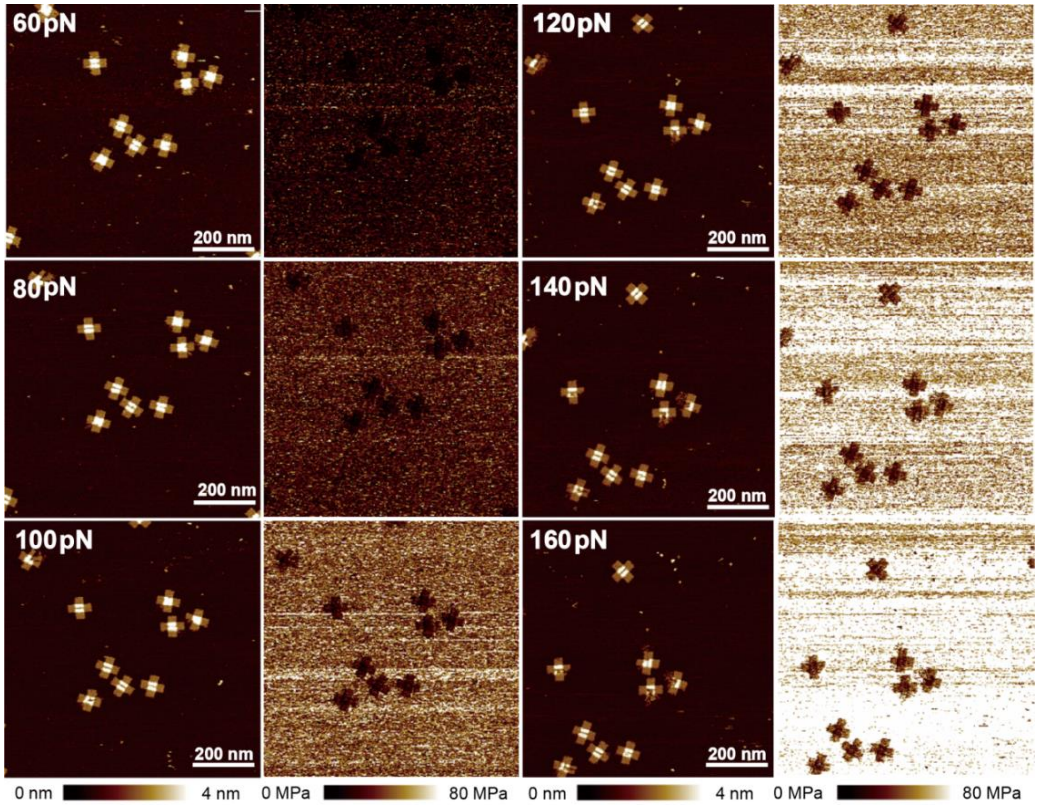

Figure S3. AFM PF-QNM images of cross-shaped DNA origamis at different peak-forces at a range of 60–160 pN. Images show the height (left) and stiffness (right) of DNA origamis.

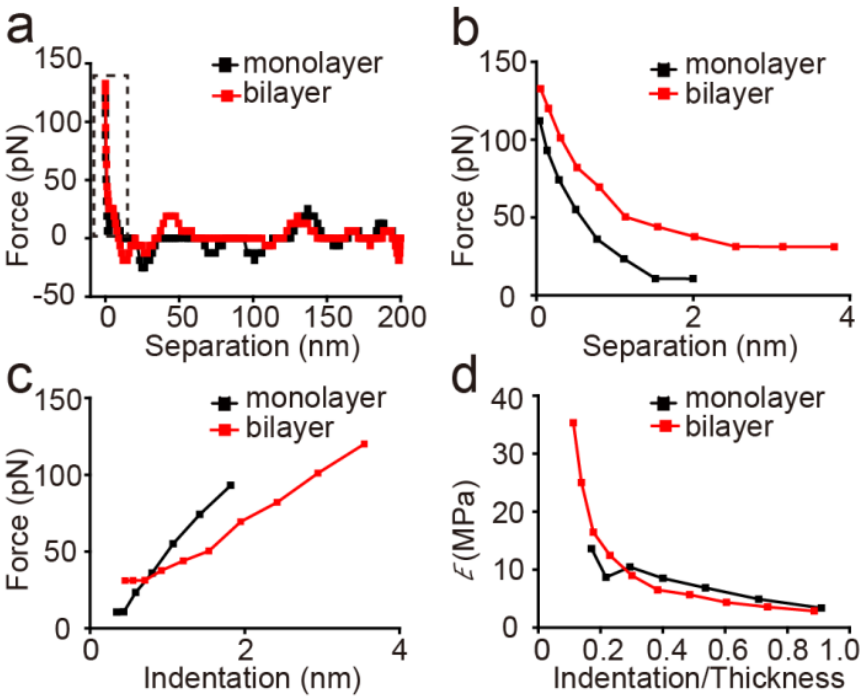

Figure S4. Reconstruction processes for the correction of Young's modulus (as described in Biophys. J. 2018, 114, 2923-29032). (a) Original approach curves of force-separation. (b) Enlarged figure marked by the rectangle in a. (c) Corresponding of the force-separation curve in b. (d) Corrected Young's modulus as a function of indentation.

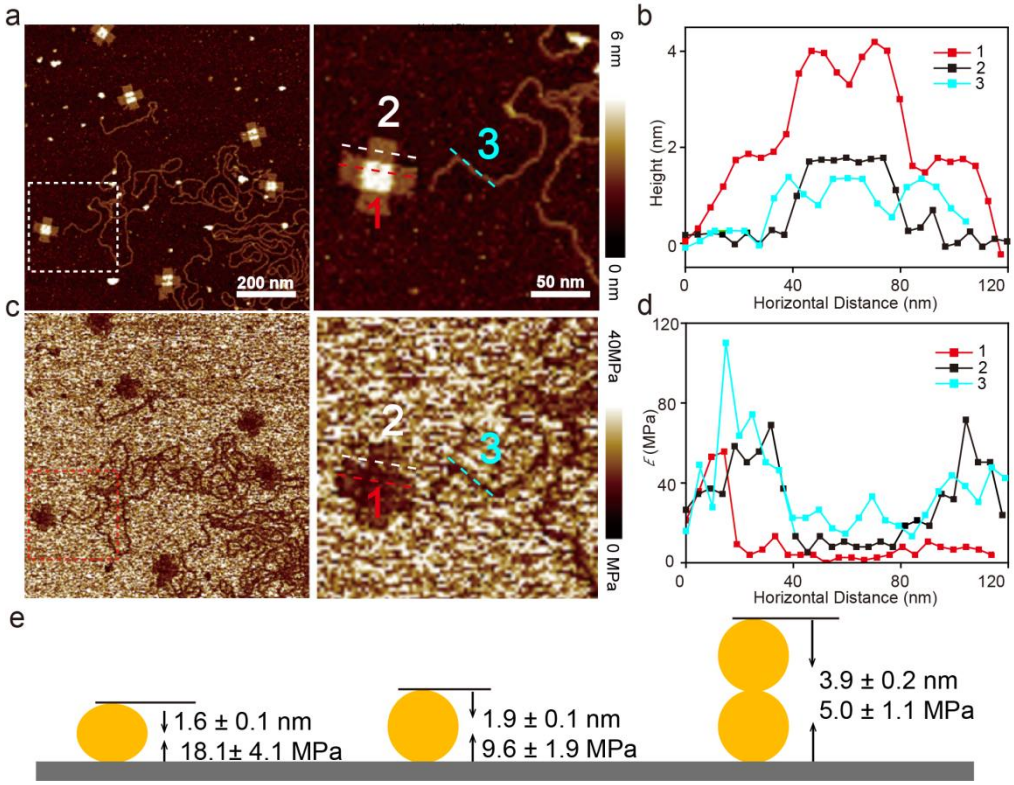

Figure S5. PF-QNM images of cross-shaped DNA origamis and lambda DNA showing the height and stiffness with a peak-force of 100 pN. (a) Topographical AFM images (left); enlarged image (right). (b) Height profile in (a, right) marked using colored lines. (c) DMT AFM images. (d) Young's modulus profile of the colored lines (c, right) showing the measured *E* of single DNA molecules, mono-, and

bilayer DNA origamis. (e) The yellow circles represent the different heights of the DNA helix in origamis (right and middle) and individual DNA molecules (left) adsorbed on the mica surface, respectively.

References

1 W. Y. Liu, H. Zhong, R. S. Wang and N. C. Seeman, *Angew Chem Int Edit.* **2011**, 50, 264-267.
2 P. W. K. Rothemund, *Nature* **2006**, 440, 297-302.
3 A. Voss, C. Dietz, A. Stocker and R. W. Stark, *Nano Res.* **2015**, 8, 1987-1996.
4 P. D. Garcia and R. Garcia, *Biophys. J.*, **2018**, 114, 2923–2932.