

on mica supported lipid bilayer

on mica supported lipid bilayer

on bare mica

 $\mathsf{e}% _{0}\left(\mathsf{e}_{0}\right)$

Supplementary Figure 1 Lattice formation on both SLB and mica surfaces. (**a**) AFM image of the mica supported lipid bilayer (SLB). (**b**) The section profile along the line A–B in (**a**). (**c,d**) AFM images after deposition of the cross-shaped DNA origami units onto the surfaces having both bare mica and SLB regions. Lattices were only observed on the bilayer surface. (**e,f**) AFM image after deposition of the cross-shaped DNA origami units onto the bare mica surface. A drop $(2 \mu L)$ of cross-shaped DNA origami nanostructures in the standard buffer (20 mM Tris buffer (pH 7.6), 1 mM EDTA, and 10 mM $MgCl₂$) solution (10 nM) was deposited onto the preformed SLB (**c,d**) or freshly cleaved mica (**e,f**) surfaces. After incubation for 60 min at room temperature, the surface was imaged by AFM in \sim 150 μ L of the standard buffer. Note that densities of origamis on bare mica regions in (**c,d**) appeared to be higher than those in (**e,f**). When origami with blunt ends was employed as an assembly unit, the stacking-interaction-mediated formation of the less-densely packed lattices were preferred rather than the close-packing ordering phenomenon. This is probably achieved by kicking out excess origami units into bulk solution or out of bilayer as observed in Fig. 2 and Supplementary Movie 1. The relatively higher density of mica-adsorbed origamis in (**c,d**) may be attributed to the accumulation of these excess units onto the bare mica regions. All scale bars are 200 nm.

 $\mathsf b$

Supplementary Figure 2 Large scale AFM images of bilayer-supported lattices made from the crossshaped DNA origamis with blunt ends. Examples of assemblies having (**a**) \sim 430, (**b**) \sim 790, and (**c**) \sim 630 origamis in the regions indicated by yellow lines. Origamis surrounded by dashed line in (**a**) was excluded from the counts. Scale bars are 400 nm.

 $\mathbf c$

(i) w/o NaCl

 $(ii) + 200$ mM NaCl

 ${\sf d}$

(ii) + 200 mM KCl

Supplementary Figure 3 Effect of the surface rinsing with NaCl. (**a**) Successive HS-AFM images of saltinduced desorption of lipid bilayer-supported lattices. While scanning of the same area is continued, 15 μL of the buffer containing 2 M NaCl was injected to 135 μL of the standard buffer (20 mM Tris buffer (pH 7.6), 1 mM EDTA, and 10 mM $MgCl₂$), so that the final concentration of NaCl was 200 mM. The lattices were desorbed from the bilayer surface immediately after this treatment. Images were obtained at a scan rate of 0.2 frames per second. (**b**) The reversible effect of NaCl. After the release of cantilever, the buffer containing 200 mM NaCl was gently sucked from the liquid cell of AFM by pipette. Then, a drop (2 μL) of the DNA origami nanostructure in the standard buffer was deposited on to the same surface. After 60 min incubation, the surface was imaged in 135 μL of the standard buffer. (**c**) The reversible effect of NaCl was also tested on the surface having both bare mica and bilayer regions. After the confirmation of the formation of lattices on the bilayer (i), the buffer for AFM imaging was changed to the buffer containing 200 mM NaCl (ii). After the release of cantilever, the buffer containing 200 mM NaCl was gently sucked from the liquid cell of AFM by pipette. Then, a drop (2 μL) of the DNA origami nanostructure in the standard buffer was deposited onto the same surface. After 60 min incubation, the surface was imaged again in 135 μL of the standard buffer (iii). (**d**) The reversible effect of KCl tested on the surface having both bare mica and bilayer regions. The experiment was performed by following the procedure in (**c**) except for the use of KCl instead of NaCl. All scale bars are 400 nm.

Supplementary Figure 4 AFM images of oligomerized cross-shaped origamis. (**a**) AFM image of crossshaped origamis at lower concentration on the bare mica surface. A drop $(2 \mu L)$ of diluted cross-shaped DNA origami nanostructures in the standard buffer solution (1 nM) was deposited onto the freshly cleaved mica surface. After incubation for 10 min at room temperature, the surface was imaged by AFM in the standard buffer. Oligomers with 2–12 origamis were observed in addition to monomers. (**b**) AFM image of cross-shaped origamis at standard concentration (10 nM) on the SLB. Image was obtained immediately after the deposition of the origami sample onto the preformed SLB. Oligomers with 3–25 origamis were observed. AFM imaging was performed in the standard buffer. Scale bars are 400 nm.

Supplementary Figure 5 Large aggregations of cross-shaped origamis with blunt ends. Cross-shaped DNA origami nanostructures in the standard buffer solution (10 nM) was incubated in a test tube for overnight $(\sim 12$ hours) at room temperature, and then deposited onto the freshly cleaved mica surface. After incubation for 10 min at room temperature, the surface was imaged by AFM in the standard buffer. Scale bars, (**a**) 400 nm and (**b**) 200 nm.

Supplementary Figure 6 *In situ* **surface modification of the assembled lattice with streptavidin.** (**a**) AFM image at 270 s in Fig. 4b. Fully-modified origamis are recognized as facing-up orientation (orange-framed). (**b**) Representative AFM images of the biotinylated lattice after treatment with streptavidin. Origamis in facing-up orientation (orange-framed) and those in facing-down orientation (blue-framed) are indicated. Statistical analysis of AFM images revealed that 47% (116/248) of the origami units had four streptavidin molecules. Among them, 95% (110/116) were recognized as having a 'facing up' orientation. Triangles indicate orientation markers. All scale bars are 100 nm.

Supplementary Figure 7 Surface modification of the cross-shaped origami with streptavidin in test tube. Representative AFM images of streptavidin-decorated cross-shaped DNA origami with T4 tails on bare a mica surface. The binding reaction was performed in 150 μL of solution containing 10 nM DNA origami, 2 μM streptavidin and 20 mM Tris buffer (pH 7.6), 1 mM EDTA, and 10 mM $MgCl₂$ for 5 min incubation at 25 °C. The mixture was deposited on to a bare mica surface and imaged in the standard buffer. High occupancy of the binding sites was achieved with a yield of 92% (435/472). Scale bars are 100 nm.

 $\mathsf b$

 $\mathbf c$

Supplementary Figure 8 Symmetric displacement of streptavidin molecules. (**a**) Time-lapse AFM images of the modification of the lattice with streptavidin molecules. While scanning of the same area was ongoing, 15 μ L of the standard buffer containing 20 μ M streptavidin was injected into 135 μ L of the imaging buffer (20 mM Tris buffer (pH 7.6), 1 mM EDTA, and 10 mM MgCl₂), so that the final concentration of streptavidin was 2 μ M. Images were obtained at a scan rate of 0.2 frames per second. The elapsed time is shown in each image. The solution of streptavidin was added at 65 s. Details are seen in Supplementary Movie 4. (**b,c**) Representative AFM images of the biotinylated lattices after the treatment with streptavidin. Yellow arrowheads indicate bound streptavidin molecules. Highly symmetric and specific placement was achieved with a 94% (1185/1256) occupancy of the binding sites. All scale bars are 100 nm.

Supplementary Figure 9 Different patterns produced by different surface properties. (**a,b**) AFM images after deposition of the stacking-prevented version of cross-shaped DNA origami units onto the surface having both bare mica and SLB regions. A drop $(2 \mu L)$ of DNA origami nanostructures in the standard buffer solution (a) : 1 nM, **b**: 10 nM) was deposited onto the surfaces. After incubation for 15 min at room temperature, the surface was imaged by AFM in the standard buffer. (**c**) Zoomed image of the lattice on the SLB region. (**d**) Zoomed image of disordered origamis on the bare mica region. Close-packing was not observed on bare mica surfaces. Scale bars, (**a,b**) 200 nm and (**c,d**) 100 nm.

Supplementary Figure 10 AFM images of DNA origami nanostructures on bare mica surfaces. A drop (2 L) of DNA origami nanostructures in the standard buffer solution (**a-c**: 1 nM, **d-i**: 10 nM) was deposited onto the freshly cleaved mica surface. After incubation for 15 min at room temperature, the surface was imaged by AFM in the standard buffer. (**a,d,g**) Cross-shaped DNA origamis, (**b,e,h**) triangular DNA origamis, and (**c,f,i**) hexagonal DNA origamis. All scale bars are 200 nm.

Supplementary Figure 11 Large scale AFM images of bilayer-supported close-packed structures. Representative large scale images of close-packed structures of (**a**) cross-shaped origamis with T4 tails, (**b**) triangular origamis, and (**c**) hexagonal origamis. Although domain boundaries of the assemblies are seen in the large scale images, micrometer-sized assemblies are routinely obtained. Scale bars are 200 nm.

Supplementary Figure 12 AFM and FFT images of the DNA origami lattices. AFM and FFT images of the lattices formed by self-assembly of (**a**) cross-shaped origamis with blunt ends, (**b**) cross-shaped origamis with T4 tails, (**c**) triangular origamis, and (**d**) hexagonal origamis. All scale bars are 200 nm.

Supplementary Figure 13 Treatment of the bilayer surface with various concentration of DNA origami nanostructures. (**a-i**) Time-lapse AFM images after incubation of the following concentrations of DNA origamis: (**a,d,g**) 1 nM, (**b,e,h**) 5 nM, and (**c,f,i**) 10 nM. Samples were prepared in the standard buffer (20 mM Tris buffer (pH 7.6), 1 mM EDTA, and 10 mM $MgCl₂$) as described in Methods section and then imaged in the same buffer. (**a-c**) Cross-shaped DNA origamis with T4 tails. (**d-f**) Triangular DNA origamis. (**g-i**) Hexagonal DNA origamis. Images were obtained at scan rate of (**a,d,g**) 1.0 or (**others**) 0.2 frames per second. Events in (**a**) and (**b**) can be seen in Supplementary Movies 6 and 7. In lower concentrations, origamis diffuse faster than the scan rate, and thus their shapes are not clearly imaged. This smearing effect is attributed to the limited scan rate of the AFM in which the tip is tracking the diffusing origamis. All scale bars are 200 nm.

Supplementary Figure 14 Feffect of Na⁺ concentration onto the close packing. Bilayer-supported closepacked structures were first prepared in the standard buffer (20 mM Tris buffer (pH 7.6), 1 mM EDTA, and 10 mM MgCl₂) as described in Methods section and then imaged in the buffer containing (**a-f**) 20 mM or (**g-l**) 100 mM NaCl (20 mM Tris buffer (pH 7.6), 1 mM EDTA, 20 mM MgCl₂, and 20 mM or 100 mM NaCl). (a-l) AFM images of the bilayer surfaces incubated with (**a,d,g,j**) cross-shaped origamis with T4 tails, (**b,e,h,k**) triangular origamis, and (**c,f,i,l**) hexagonal origamis. (**a-c**) AFM images of the bilayer surfaces in the buffer containing 20 mM NaCl. (**d-f**) Time-lapse AFM images of the bilayer surfaces in the presence of 20 mM NaCl. Images were obtained at scan rate of 0.2 frames per second. (**g-i**) AFM images of the bilayer surfaces in the presence of 100 mM NaCl. Origamis were not observed under this condition. (**j-l**) The same experiments with (**g-i**) were performed using the surfaces having both bare mica and SLB regions. Origamis were observed solely on bare mica regions. All scale bars are 200 nm.

Supplementary Figure 15 NaCl induced desorption of origami structures from mica/SLB surfaces. A drop (2 μ L) of cross-shaped DNA origamis with T4 tails in the standard buffer solution (10 nM) was deposited onto the surfaces having both bare mica and SLB regions. After incubation for 15 min at room temperature, the surface was imaged by AFM in the buffer containing (**a**) 0 mM, (**b**) 20 mM, (**c**) 100 mM, and (**d**) 200 mM of NaCl. Scale bars are 400 nm.

Supplementary Figure 16 \vert Effect of decrease in Mg²⁺ concentration ([MgCl₂]=5 mM) on the close packing. Bilayer-supported close-packed structures were first prepared in the standard buffer (20 mM Tris buffer (pH 7.6), 1 mM EDTA, and 10 mM $MgCl₂$) as described in Methods section and then imaged in low $[MgCl₂]$ buffer (20 mM Tris buffer (pH 7.6), 1 mM EDTA, and 5 mM MgCl₂). (a-f) AFM images of the bilayer surfaces incubated with (**a,b**) cross-shaped origamis with T4 tails, (**c,d**) triangular origamis, and (**e,f**) hexagonal origamis. (**g-i**) Timelapse AFM images of the bilayer surfaces incubated with (**g**) cross-shaped origamis with T4 tails, (**h**) triangular origamis, and (**i**) hexagonal origamis. Images were obtained at scan rate of 0.5 frames per second. All scale bars are 200 nm.

Supplementary Figure 17 | Effect of increase in Mg^{2+} concentration ([MgCl₂]=20 mM) onto the close **packing.** Bilayer-supported close-packed structures were first prepared in the standard buffer (20 mM Tris buffer (pH 7.6), 1 mM EDTA, and 10 mM MgCl₂) as described in Methods section and then imaged in high $[Mg^{2+}]$ buffer (20 mM Tris buffer (pH 7.6), 1 mM EDTA, and 20 mM MgCl₂). (a-f) AFM images of the bilayer surfaces incubated with (**a,b**) cross-shaped origamis with T4 tails, (**c,d**) triangular origamis, and (**e, f**) hexagonal origamis. (**g-i**) Time-lapse AFM images of the bilayer surfaces incubated with (**g**) cross-shaped origamis with T4 tails, (**h**) triangular origamis, and (**i**) hexagonal origamis. Images were obtained at scan rate of 0.2 frames per second. All scale bars are 200 nm.

Supplementary Table 1 Staple DNA strands for the cross-shaped DNA origami structure.

Supplementary Table 2 Staple DNA strands for preventing stacking interaction.

Supplementary Table 3 Staple DNA strands with biotin for streptavidin labeling.

Supplementary Table 4 Staple DNA strands with biotin for periodic positioning of streptavidin.

