Supporting Information

Lock-and-key mechanism for the controllable fabrication of DNA origami structures

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EXPERIMENTAL SECTION

Chemicals and Reagents. Tris-HCl, EDTA and $MgCl_2$ were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Polyvinyl sulphonic acid was purchased from Sigma-Aldrich Co. Single-stranded M13mp18 ssDNA was obtained from New England Biolabs, Inc. (Ipswich, MA, catalog no. N4040S). The staple strands for the fabrication of the DNA origami were received from Sigma Genosys (Hokkaido, Japan). The gel-filtration column and sephacryl S-500 were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA) and GE Healthcare UK Ltd. (Buckinghamshire, UK), respectively. Water was deionized (≥18.0 MΩ cm specific resistance at 25 °C) by a Milli-Q system (Millipore Corp., Bedford, MA). Cationic comb-type copolymers were prepared and purified as we have described previously (Maruyama et al. *Bioconjugate Chem.*, **1997**, *8*, 3-6; and Maruyama et al. *Bioconjugate Chem.*, **1998**, *9*, 292-299).

Design of the DNA Origami. In the present study, we used the jigsaw-shaped DNA origami (JP-1) that we have designed previously. It is a 24-helix tile with the size of ~100 nm × 80 nm. The detailed design of JP-1 and the sequences of the staple strands are given in Supporting Information of (Rajendran et al. *ACS Nano*, **2011**, *5*, 665-671). The outline of the JP-1 origami structure is also given in Figure S1.

Lock-and-Key Mechanism for the Preparation of Origami. The origami structure was prepared by following the procedure originally described by Rothemund (Rothemund, P. W. K. *Nature* **2006**, *440*, 297). For a step-by-step procedure for the preparation and purification of "Rothemund's scaffolded DNA origami structures", see our recent protocol (Rajendran et al. *Curr. Protoc. Nucleic Acid Chem.* **2012**, *48*, 12.9.1). The lock-and-key mechanism for the controllable fabrication of origami adopts the following procedures:

a) Lock M13mp18 and release using PVS:

i) M13mp18 ssDNA (final concentration of 10 nM) was mixed with 1x Tris-HCl buffer (final concentration: 20 mM Tris-HCl, pH 7.6, 10 mM $MgCl_2$ and 1 mM EDTA) and CCC (with desired N/P ratio)

ii) The above solution mixture was incubated at room temperature for 30 min, so that the CCC can sufficiently bind to the M13mp18

iii) Staple DNA strands (4 equiv., 40 nM) and PVS (5 times higher concentration than that of the CCC) was added to the solution
iv) The solution was annealed from 85ºC to 15ºC (-1ºC/min)
v) The solution was purified using sephacryl S-500 gel filtration column (prepared in 1x Tris-HCl buffer) and imaged using high-speed atomic force microscopy (HS-AFM)

b) Lock staple strands and release using PVS:

i) Staple strands (final concentration of 40 nM) was mixed with 1x Tris-HCl buffer and CCC (with desired N/P ratio)
ii) The above solution mixture was incubated at room temperature for 30 min, so that the CCC can sufficiently bind to the staple strands
iii) M13mp18 (final concentration of 10 nM) and PVS (5 times higher concentration than that of the CCC) was added to the solution
iv) The solution was annealed from 85ºC to 15ºC (-1ºC/min)
v) The solution was purified using sephacryl S-500 gel filtration column and imaged using HS-AFM

c) Lock both M13mp18 and staple strands, and release using PVS:

i) M13mp18 (final concentration of 10 nM) was mixed with staple strands (4 equiv., 40 nM), 1x Tris-HCl buffer and CCC (with desired N/P ratio)
ii) The above solution mixture was incubated at room temperature for 30 min, so that the CCC can sufficiently bind to M13mp18 and staple strands
iii) PVS (5 times higher concentration than that of the CCC) was added to the solution
iv) The solution was annealed from 85ºC to 15ºC (-1ºC/min)
v) The solution was purified using sephacryl S-500 gel filtration column and imaged using HS-AFM

Control experiments:

For all three methods mentioned above, we have also performed control experiments in which all the experimental steps are same as above while no PVS was added.

Experiments in the presence of phosphate buffer:

Instead of Tris-HCl, phosphate buffer (with the mentioned concentrations) was used in all the three methods mentioned above. In this case no PVS was added.

Experiments in the presence of mononucleotide triphosphates (NTPs):

In all the three methods mentioned above, NTPs (with the mentioned concentrations) were used instead of PVS.

N/P ratio is defined as:

N/P ratio = $\{[\text{amino groups}]_{\text{copolymer}}/[\text{phosphate groups}]_{\text{DNA}}\}$
where, DNA = M13mp18 + staple strands

AFM Imaging. AFM images were recorded using a fast-scanning AFM system (Nano Live Vision, RIBM Co. Ltd., Tsukuba, Japan) with a silicon nitride cantilever (resonant frequency 1.0-2.0 MHz, spring constant 0.1-0.3 N/m, EBDTip radius <15 nm, Olympus BL-AC10EGS-A2). The sample (2 μL) was adsorbed onto a freshly cleaved mica plate (ϕ 1.5 mm, RIBM Co. Ltd., Tsukuba, Japan) for 5 min at room temperature and then the surface was gently washed 3-5 times using 1x Tris-HCl buffer solution. Scanning was performed by tapping mode in the same buffer solution. All images reported here were recorded with an image acquisition speed of 0.2 frame/s.

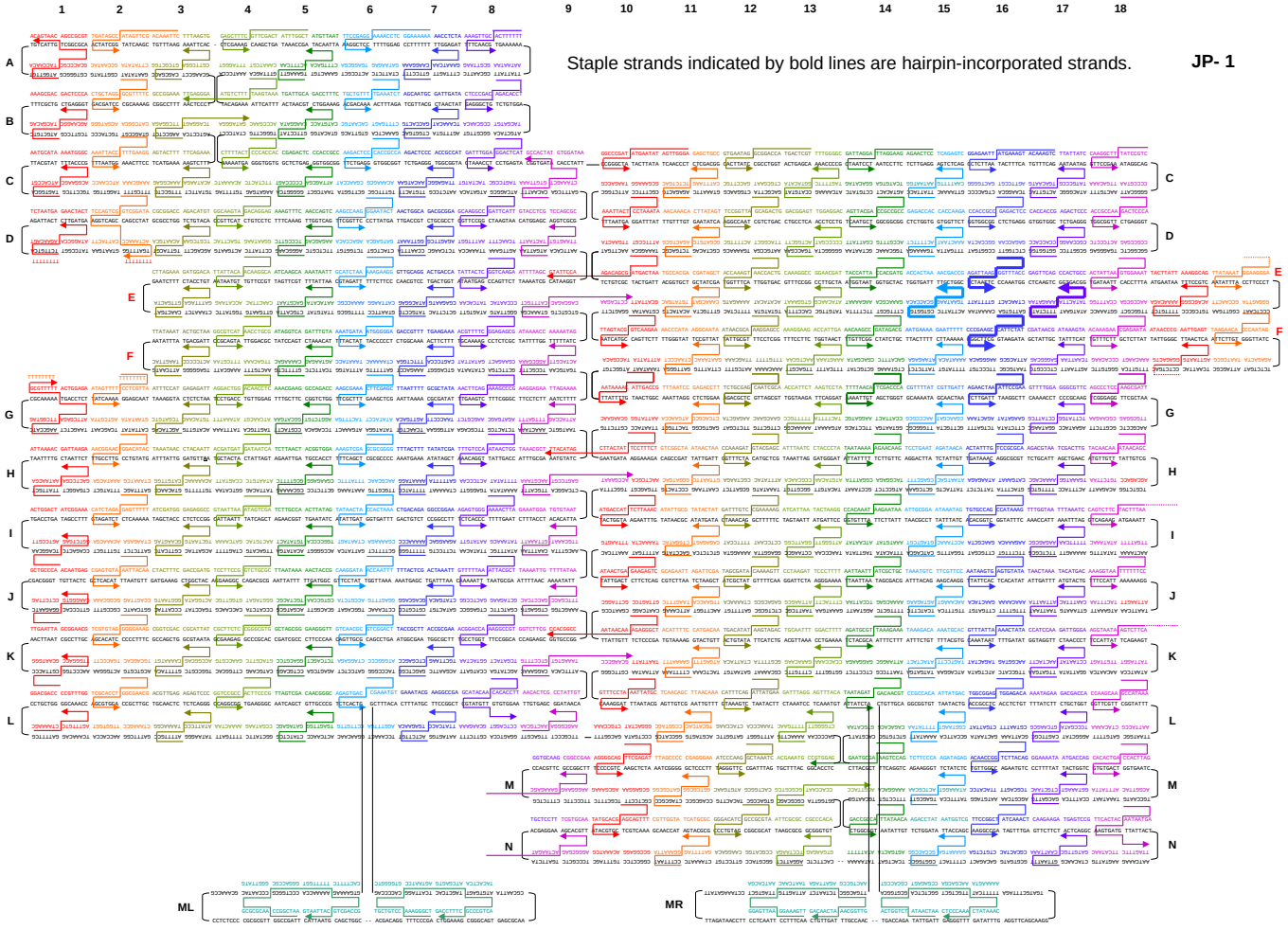


Fig. S1. The design of the jigsaw-shaped DNA origami (JP-1) which is used in this study.

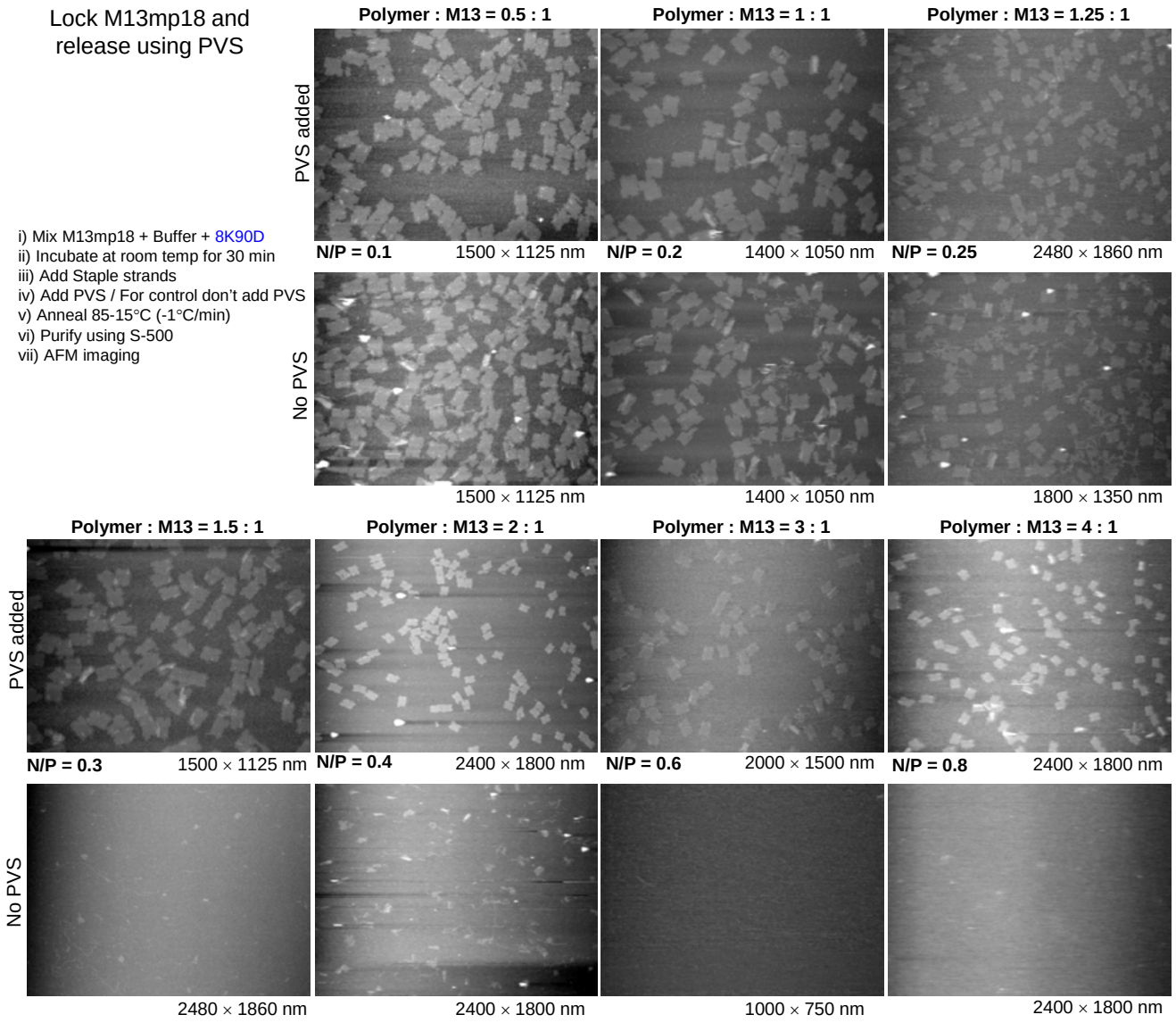


Fig. S2. AFM images of the DNA origami formation when M13 DNA was locked using 8K90D and either released using PVS (sample) or no PVS was used (control). The experiments were performed with different N/P ratio. Image sizes are given at the bottom right corner of each image. [M13mp18] = 10 nM; [Staples] = 40 nM; [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; Polymer : PVS = 1 : 5; Annealing: 85-15ºC (-1ºC/min).

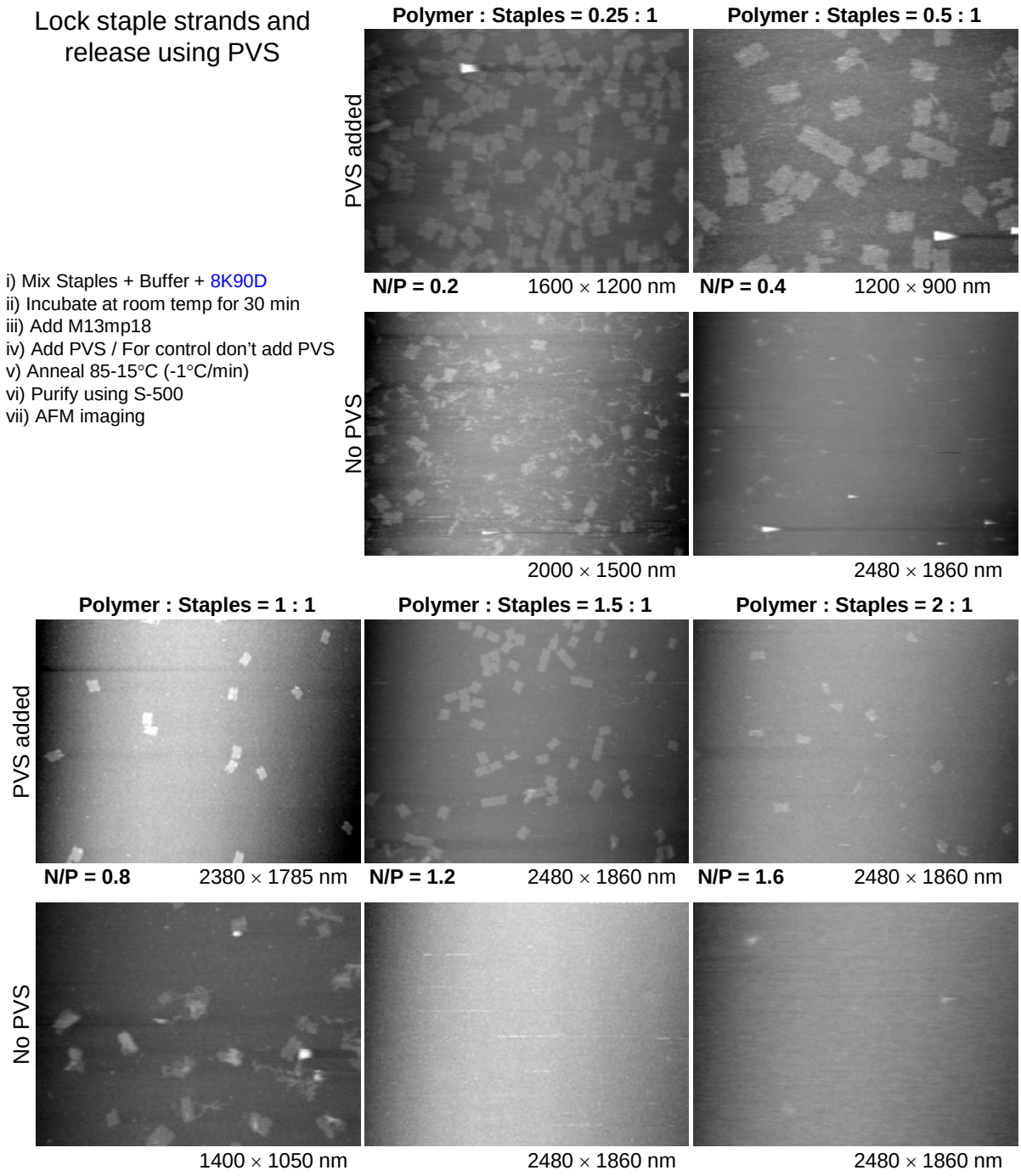


Fig. S3. AFM images of the DNA origami formation when staple strands were locked using 8K90D and either released using PVS (sample) or no PVS was used (control). The experiments were performed with different N/P ratio. Image sizes are given at the bottom right corner of each image. [M13mp18] = 10 nM; [Staples] = 40 nM; [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; Polymer : PVS = 1 : 5; Annealing: 85-15ºC (-1ºC/min).

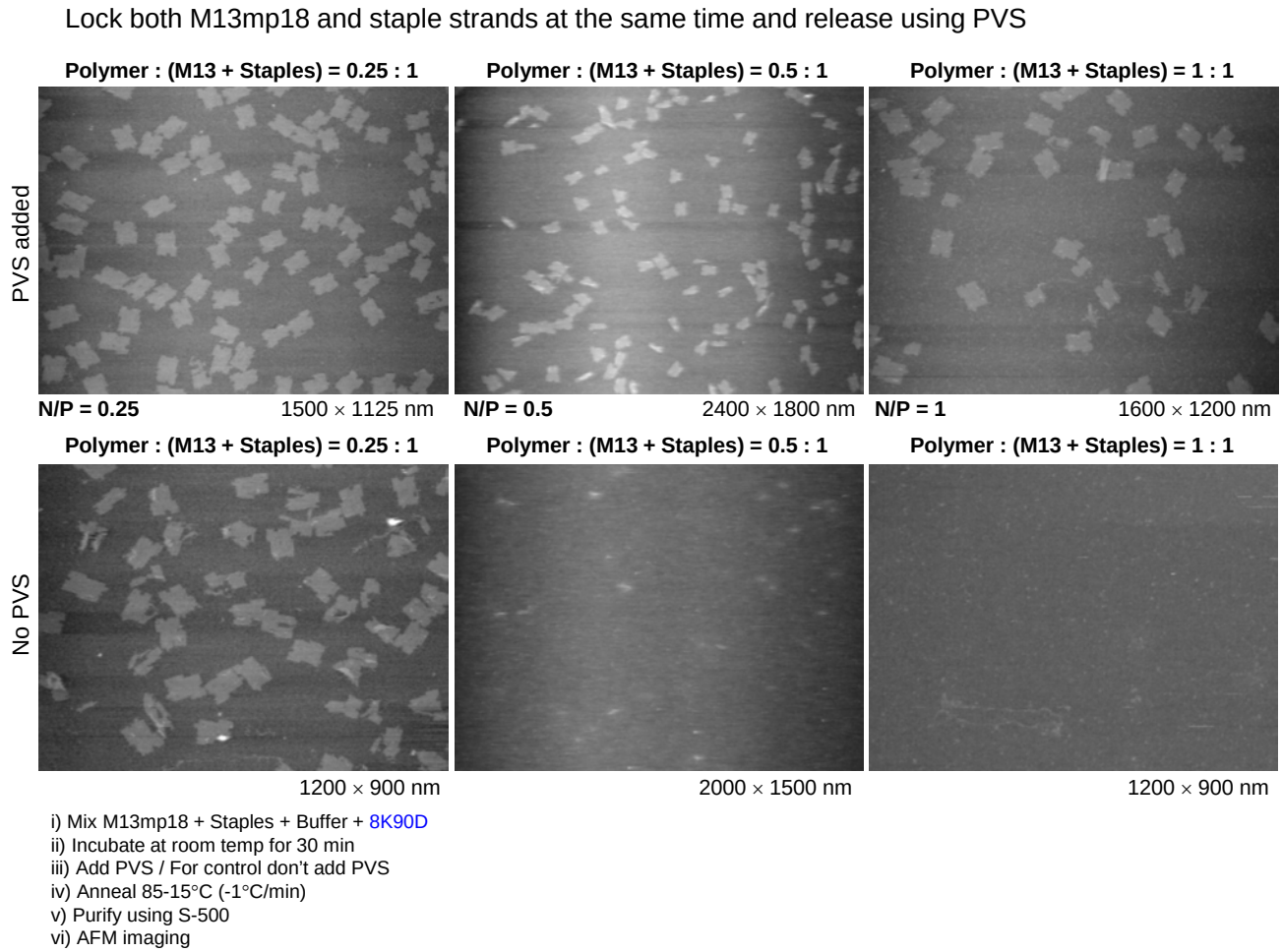
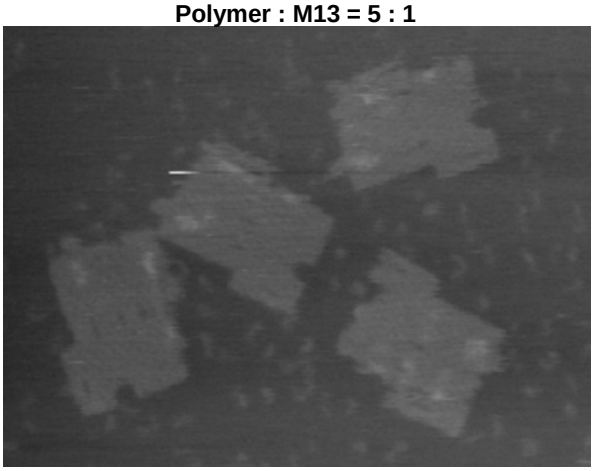


Fig. S4. AFM images of the DNA origami formation when both M13 and staples were locked using 8K90D and either released using PVS (sample) or no PVS was used (control). The experiments were performed with different N/P ratio. Image sizes are given at the bottom right corner of each image. [M13mp18] = 10 nM; [Staples] = 40 nM; [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; Polymer : PVS = 1 : 5; Annealing: 85-15ºC (-1ºC/min).

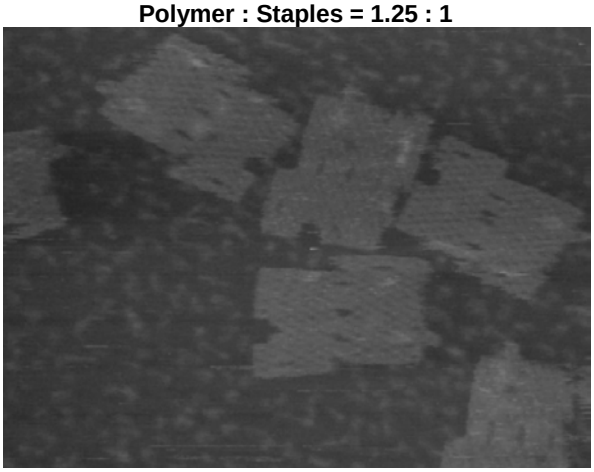
Lock M13

Polymer : M13 = 5 : 1



Lock staples

Polymer : Staples = 1.25 : 1



Lock both M13 and staples

Polymer : (M13 + Staples) = 1 : 1

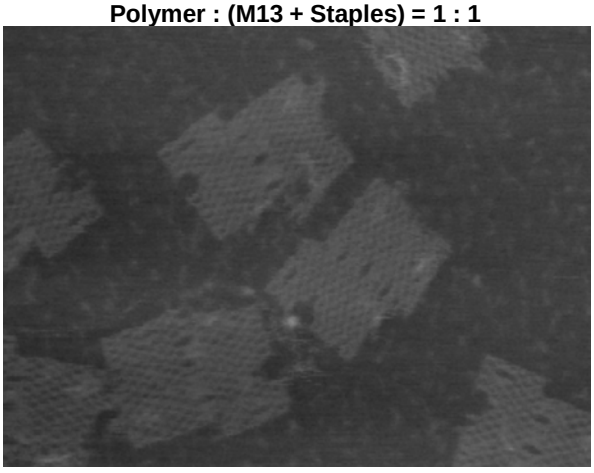


Fig. S5. Representative zoom-in AFM images of the DNA origami formation when ssDNAs were locked using 15K92D and released using PVS. All experimental conditions are same as given in main text Fig. 3. Image size: 400 nm × 300 nm.

Table S1. The yield of the jigsaw-shaped origami structure at different conditions

Polymer	Lock	Polymer:DNA	N/P Ratio	Yield	Number of Tiles Calculated
8K90D	M13	2:1	0.4	90%	202
8K90D	Staples	0.5:1	0.4	86%	165
8K90D	Both	0.5:1	0.5	72%	79
15K92D	M13	5:1	1	95%	144
15K92D	Staples	1.25:1	1	90%	208
15K92D	Both	1:1	1	86%	234

All experimental conditions are same as given in main text Fig. 2 and 3.

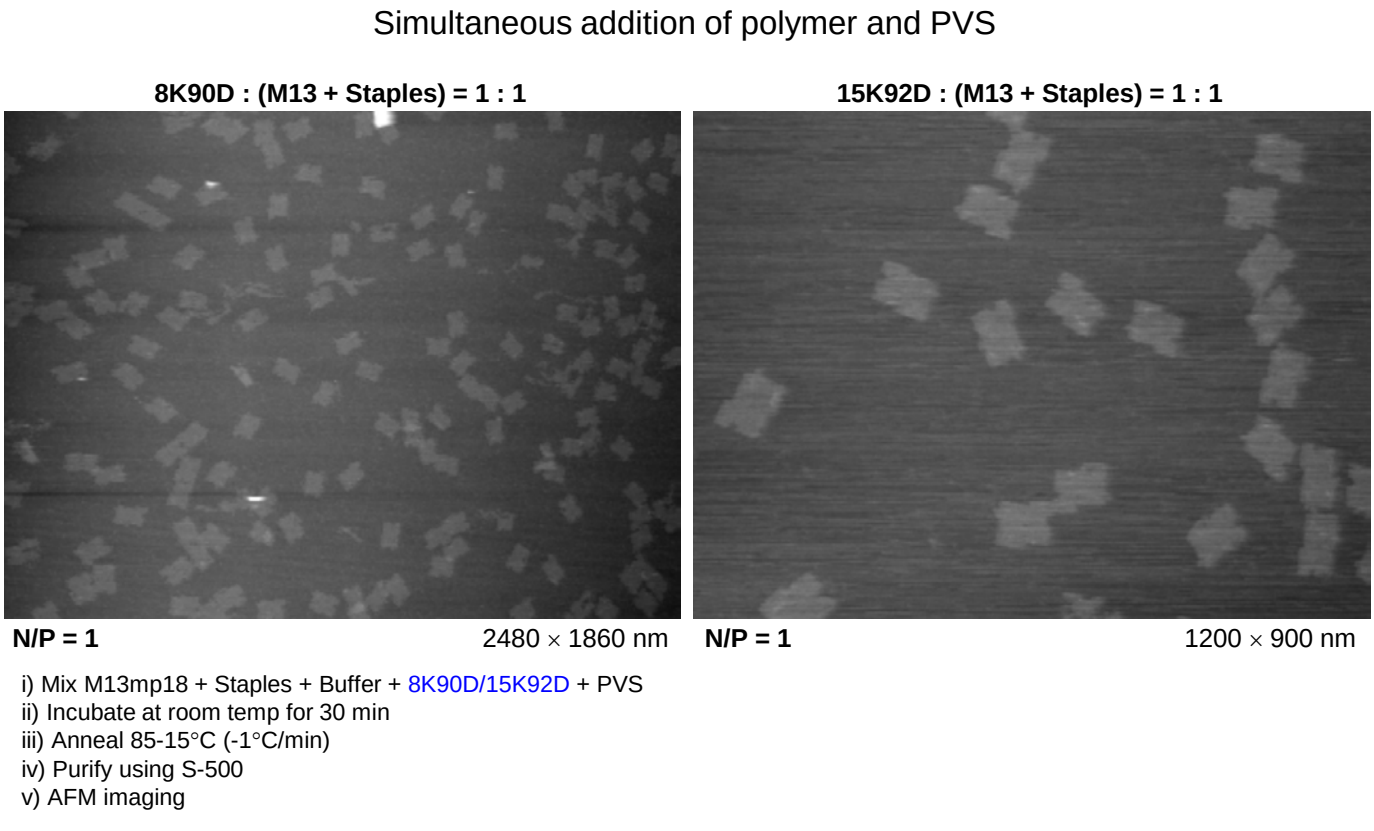
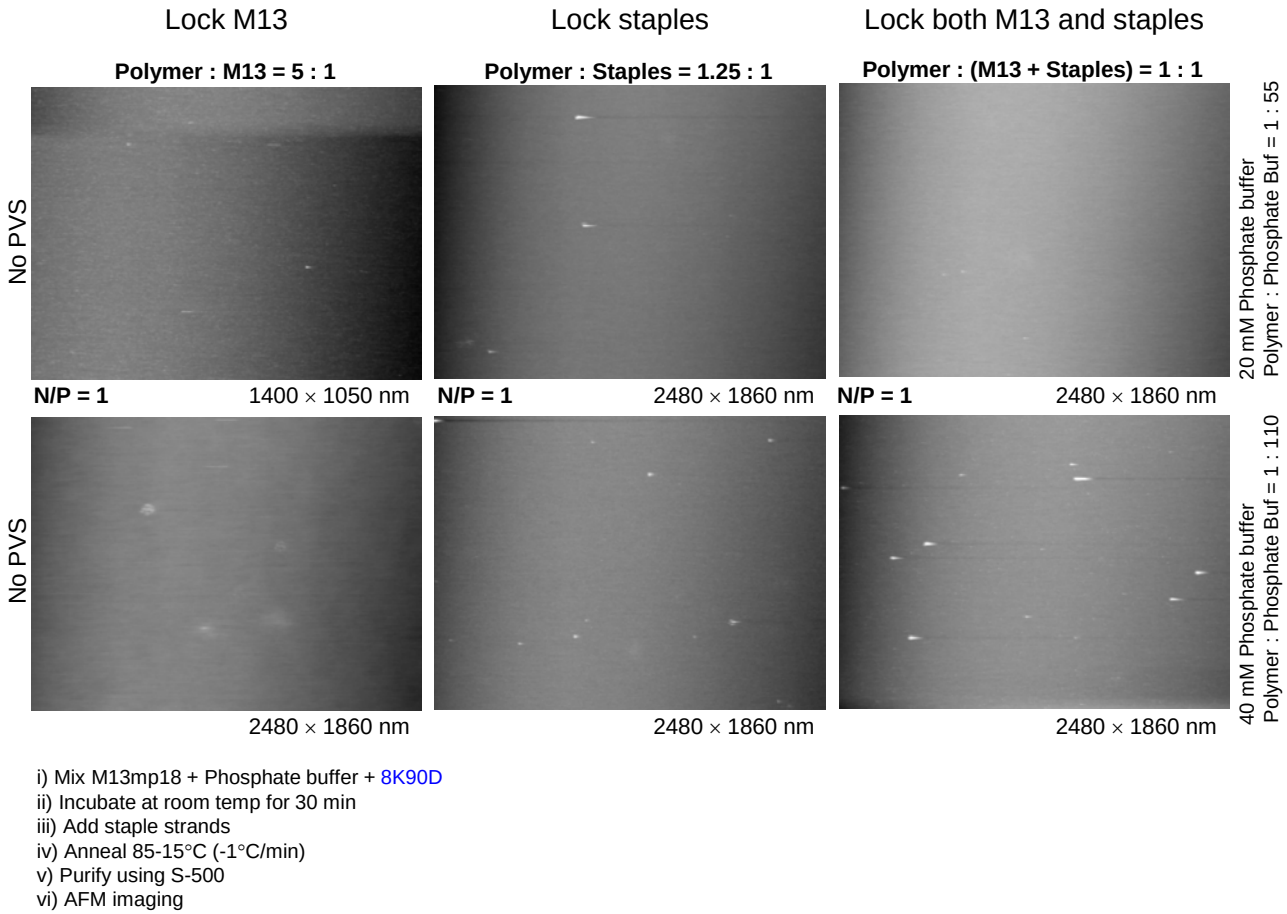
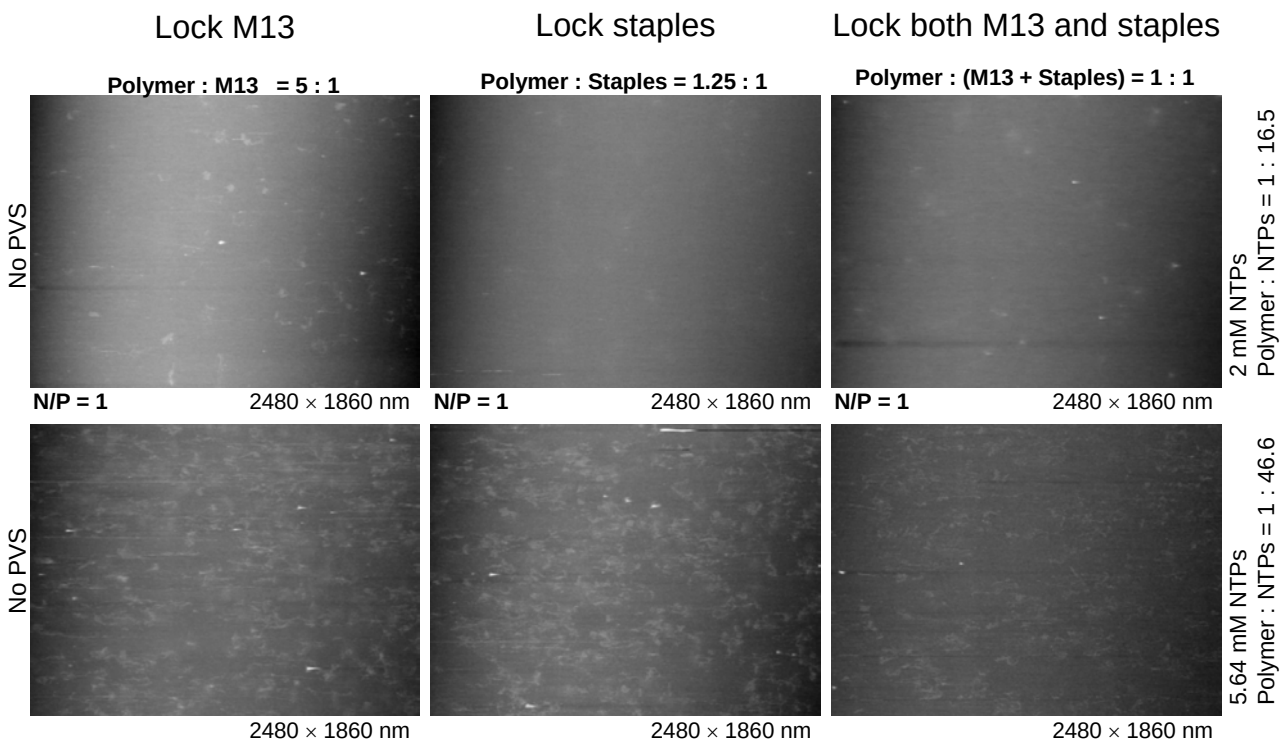


Fig. S6. AFM images of the DNA origami formation when CCC (left: 8K90D and right: 15K92D) and PVS were simultaneously added. Image sizes are given at the bottom right corner of each image. [M13mp18] = 10 nM; [Staples] = 40 nM; [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; N/P = 1; Polymer : PVS = 1 : 5; Annealing: 85-15ºC (-1ºC/min).



i) Mix M13mp18 + Phosphate buffer + 8K90D
ii) Incubate at room temp for 30 min
iii) Add staple strands
iv) Anneal 85-15°C (-1°C/min)
v) Purify using S-500
vi) AFM imaging

Fig. S7. AFM images of the DNA origami formation when either M13/staples alone or both together locked using 8K90D and the experiments were performed in phosphate buffer. No PVS was added in these experiments. Image sizes are given at the bottom right corner of each image. [M13mp18] = 10 nM; [Staples] = 40 nM; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; Buffer pH 7.6; N/P = 1; Annealing: 85-15ºC (-1ºC/min).



i) Mix M13mp18 + Buffer + NTPs + 8K90D
ii) Incubate at room temp for 30 min
iii) Add staple strands
iv) Anneal 85-15°C (-1°C/min)
v) Purify using S-500
vi) AFM imaging

Fig. S8. AFM images of the DNA origami formation when either M13/staples alone or both together locked using 8K90D and the experiments were performed in the presence of NTPs. No PVS was added in these experiments. Image sizes are given at the bottom right corner of each image. [M13mp18] = 10 nM; [Staples] = 40 nM; [Tris-HCl] = 20 mM, pH 7.6; [$MgCl_2$] = 10 mM; [EDTA] = 1 mM; N/P = 1; Annealing: 85-15ºC (-1ºC/min).