Electronic Supplementary Material (ESI) for Nanoscale.


# Surface-Assisted Self-Assembly of 2D, DNA Binary Crystals

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# Supporting Information

**Materials and Experimental Methods**

**Oligonucleotides.** DNA sequences have been designed by a computer program "SEQUIN" (Seeman, N. C. *J. Biomol. Struct. Dyn.* 1990, *8*, 573-581). All oligonucleotides were purchased from IDT Inc., purified by 6% - 20% denaturing PAGE, and their concentrations were quantified by UV-Vis spectroscopy at 260 nm. Oligonucleotides from b5PS, and 5PS motifs are listed below with their stoichiometric molar ratio and concentration during *in situ* assembly.

***DNA Strands:***

**L1**: 5'-AGGCACCATCGTAGGTTTCTTGCCAGGCACCATCGTAGGTTTCTTGCCA GGCACCATCGTAGGTTTCTTGCC-3'

**L2**: 5'-AGGCACCATCGTAGGTTTTCTTGCCAGGCACCATCGTAGGTTTTCTTGCC AGGCACCATCGTAGGTTTTCTTGCCAGGCACCATCGTAGGTTTTCTTGCCA GGCACCATCGTAGGTTTTCTTGCCAGGCACCATCGTAGGTTTTCTTGCC-3' ( circular by ligation at 5' and 3' ends)

**M1**: 5'-GACTATGCAACCTGCCTGGCAAGCCTACGATGGACACGGTAACGC-3'

**M2**: 5'-GACTATGCAACCTGATACCCTTAGTATGTAGCCTGCCTGGCAAGCCTAC GATGGACAATCTATTATGCGATTCGGACACGGTAACGC-3'

**J1**: 5'-TATCACCGAATCGCATAATAG-3'

**J2**: 5'-ATTGTGGCTACATACTAAGGG-3'

**J1b**: 5'-TATCACCGAATCGCATAATAGCGTCGAACG-3'

**J2b**: 5'-ATTGTGGCTACATACTAAGGGCGTCGAACG-3'

**S1**: 5'-AGGCGTTACCGTGTGGTTGCATAGTCAG-3'

**S2**: 5'-CTGCGTTACCGTGTGGTTGCATAGTCCT-3'

**BB**: 5'-CATGAAGCTATAGCTTCATGCGTTCGACT-3'

***DNA Motif:***

**3PS**: L1+M1+S1 (1:3:3, 1 μM).

**6PS**: L2+M1+S2 (1:6:6, 400 nM).

**e3PS**: L1+M2+J1+J2+S1 (1:3:3:3:3, 400 nM).

**b3PS**: L1+M2+J1b+J2b+S1+BB (1:3:3:3:3:6, 400 nM).

**Buffers.**

**TA/ $Mg^{2+}$ buffer**: 40 mM tris base, 20 mM acetic acid, and 10 mM magnesium acetate; pH is adjusted to 8.0

**TAE/$Mg^{2+}$ buffer**: 40 mM tris base, 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate; pH is adjusted to 8.0

**TA/Na/*x* mM $Mg^{2+}$ buffer**: 40 mM tris base, 20 mM acetic acid, designated *x* mM magnesium acetate, and 500 mM sodium chloride; pH is adjusted to 8.0

**TA/$Mg^{2+}$/$Ni^{2+}$ buffer**: 40 mM tris base, 20 mM acetic acid, 10 mM magnesium acetate, and 2 mM nickel chloride; pH is adjusted to 8.0

**Preparation of individual DNA motifs**. (1) For 3PS, e3PS, and 6PS, mix all ssDNAs (except **S1** or **S2**) at designated stoichiometric molar ratio in TA/$Mg^{2+}$ solution. Sequentially incubate above solutions: 95 °C for 5 min, 65 °C for 30 min, 50 °C for 30 min, 37 °C for 30 min, and 22 °C for 30 min. Then add **S1** or **S2** at designated ratio to a final 40 μL TA/$Mg^{2+}$ solution. Sequentially incubate: 37 °C for 30 min, 22 °C for 30 min. (2) For b3PS motif, mix all ssDNAs (except **S1** and

bridge strands **BB**) at designated stoichiometric molar ratio in TA/$Mg^{2+}$ solution. Sequentially incubate above solutions: 95 °C for 5 min, 65 °C for 30 min, 50 °C for 30 min, 37 °C for 30 min, and 22 °C for 30 min. Then add **S1** at designated ratio to a final 40 μL TA/$Mg^{2+}$ solution [500 nM motif solution (no bridges)]. Sequentially incubate: 37 °C for 30 min, 22 °C for 30 min. Add 10 μL of bridge DNA, **BB**, into the annealed solution above and incubate for 1 hour at 22 °C [final 50 μL 400 nM b3PS motif solution (with bridges)].

**Native PAGE**. Mix 40% acrylamide/bisacrylamide solution (19:1, 5% crosslinker), 10× TAE/$Mg^{2+}$ buffer, and distilled water to prepare 4% or 6% native PAGE gel. The running buffer was TAE/$Mg^{2+}$ buffer (containing 40 mM tris base, 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate; pH is adjusted to 8.0). Gels were run on a FB-VE10-1 electrophoresis unit (FisherBiotech) at 4 °C (300V, constant voltage) for 2 hours. After electrophoresis, the gels were stained with Stains-all dye (Sigma) and scanned.

**Surface assembly and AFM imaging of DNA arrays.** (1) Preparation: Mix 3PS (3PS, e3PS, or b3PS) and 6PS motif at designated motif ratio to a final 150 nM or 200 nM DNA motif in TA/Na/$Mg^{2+}$ (50 mM $Mg^{2+}$ for 3PS, 22.5 mM or 30 mM $Mg^{2+}$ for e3PS and b3PS) solution. One exception is 3PS:6PS=10:1, in which [3PS]=400 nM and [6PS]=40 nM. (2) Surface assembly: Deposit 5 μL DNA solution onto a freshly cleaved mica surface and incubate for 24 to 96 hours at 22 °C for array formation. Note that the formation of the binary arrays may undergo tile recognition and displacement thus take longer time to assemble (K. Tapoi et al., *Chem. Mater*., 2023, **35**, 1961–1971; Y. Xin et al., *Chem. Eur. J.*, 2021, **27**, 8564–8571; C. Kielar et al., *ACS Appl. Mater. Interfaces*, 2018, **10**, 44844–44853). And little change is observed after 24-hour incubation. (3) Buffer wash: After surface assembly, 20 μL TA/Na/100 mM $Mg^{2+}$ buffer is added onto mica surface and then removed. Repeat this process for four times. (4) AFM image capture: 20 μL TA/$Mg^{2+}$/$Ni^{2+}$ buffer is added onto mica surface. Then AFM images are captured by MultiMode 8 (Bruker) using ScanAsyst-fluid mode with ScanAsyst-fluid+ probes (Bruker). The tip-surface interaction was automatically adjusted to optimize the scan set-point. All experiments are carried out at 22 °C.

**FFT (Fast Fourier Transform) and inverse FFT processing:** Import the original AFM images into ImageJ software (developed by the National Institutes of Health, Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018). Use FFT operation to obtain FFT diffraction patterns then followed by brightness-and-contrast adjustment. Pick up the targeted intense diffraction spots and use inverse FFT operation to render reconstructed images.

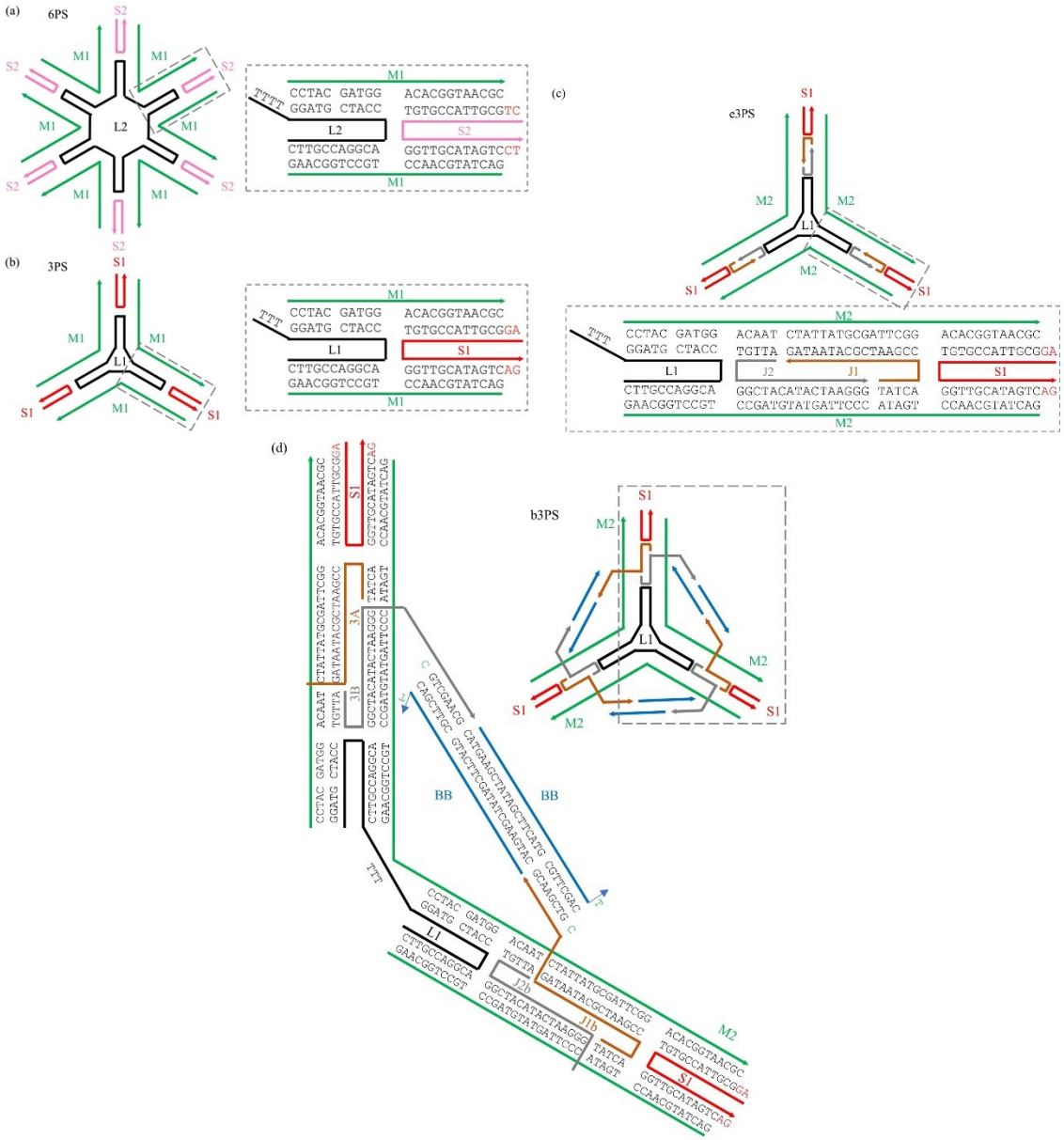


**Figure S1.** Schemes of symmetric, point-star motifs: (a) 6PS, (b) 3PS, (c) e3PS, and (d) b3PS. Each motif has either a 3- or 6-fold rotational symmetry, which renders that all the branches and bridges (for b3PS) are identical in terms of both structure and DNA sequences. Detailed view of one branch and bridge (for b3PS) are shown in dashed rectangular box. Each branch contains a pair of 2-nt-long sticky ends (sequence colored red). The duplex bridges between the branches in b3PS contain 1 unpaired nucleotide at both ends (sequence colored green).

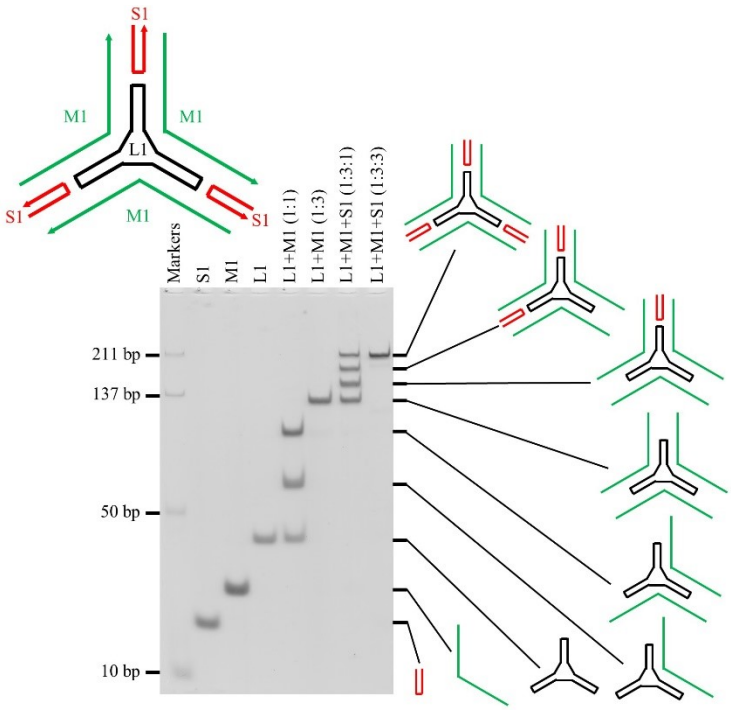


**Figure S2.** PAGE (8%) analysis of the formation of 3PS (Structural scheme shown at the upper left). The sample compositions and the band identifiers are indicated above and at the sides of the gel image, respectively.

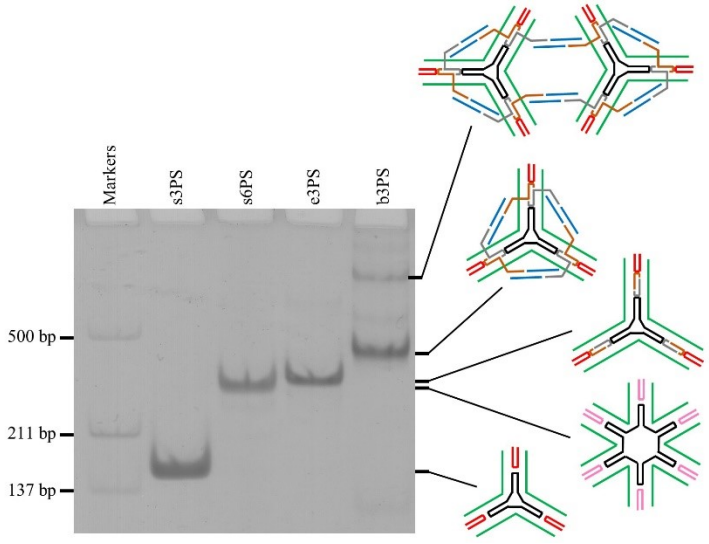


**Figure S3.** PAGE (4%) analysis of DNA motifs: 3PS, 6PS, e3PS, and b3PS. The sample compositions and the band identifiers are indicated above and at the sides of the gel image, respectively.

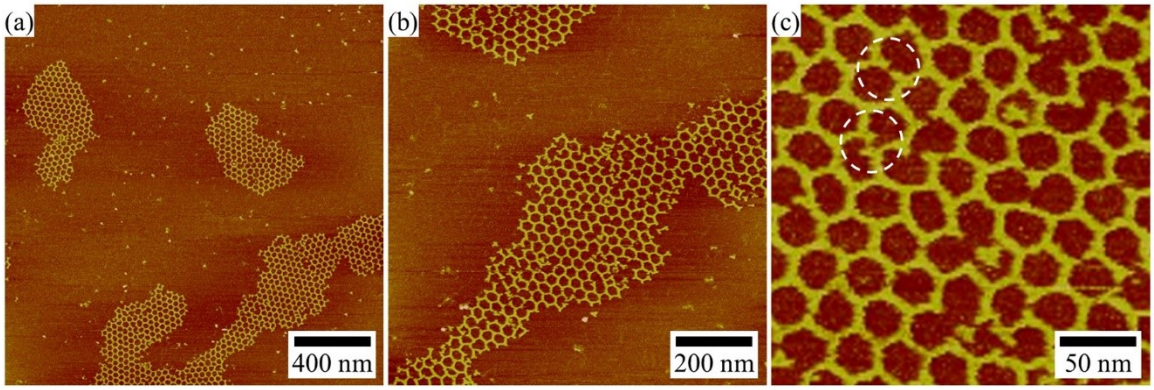


**Figure S4.** Fragmented arrays assembled from 3PS. (a-c) A set of AFM images at different magnifications of the DNA arrays. The white circles highlight the broken inter-motif bonds.

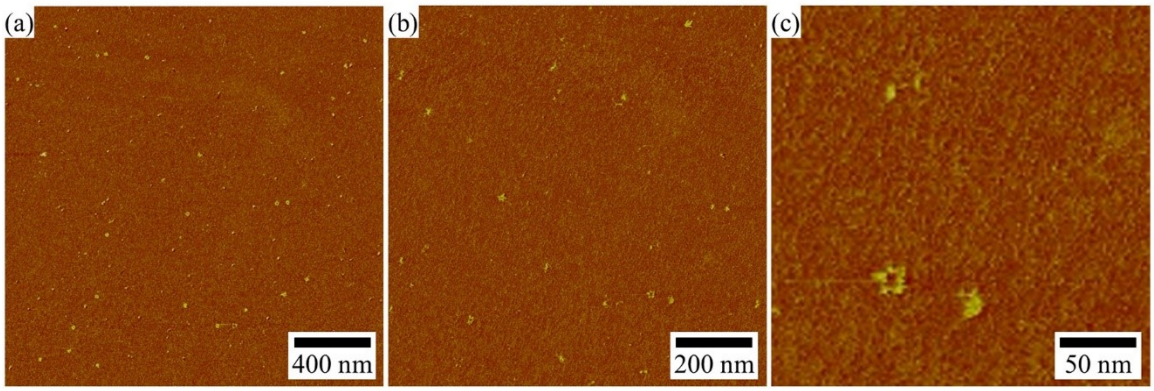


**Figure S5.** No array observed for 6PS. (a-c) A set of AFM images at different magnifications.

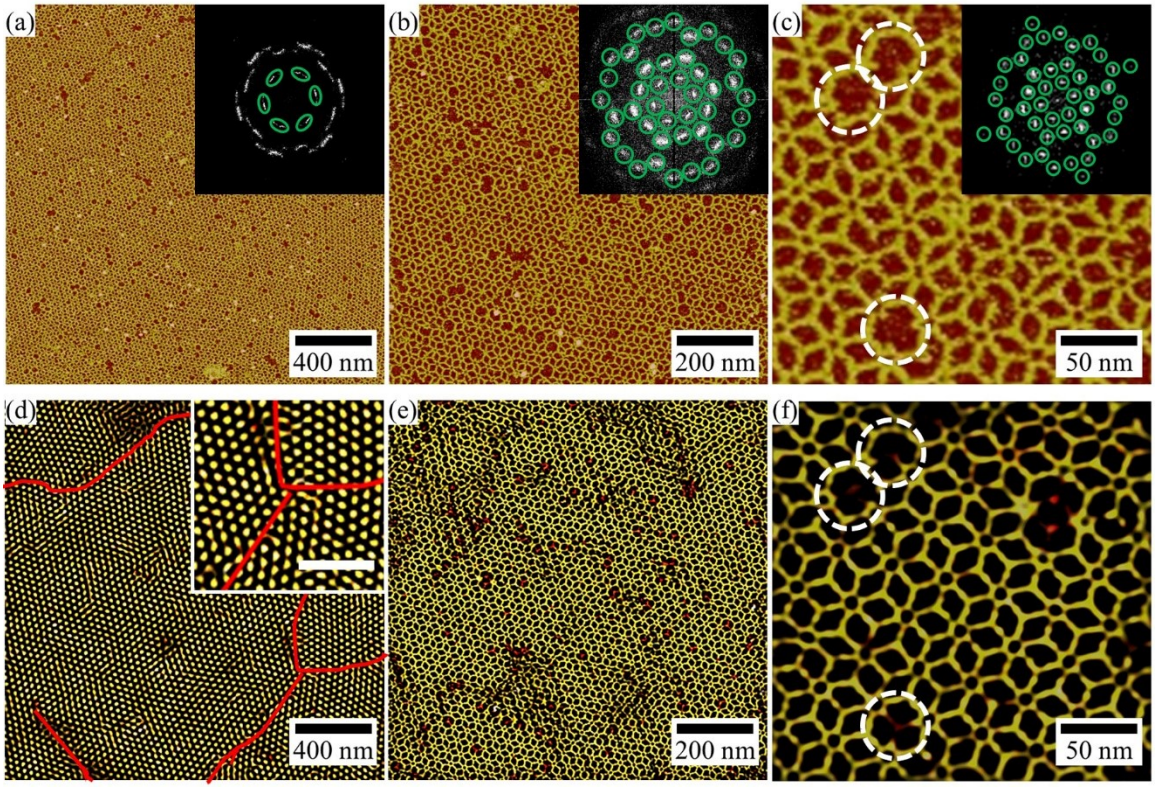


**Figure S6.** Binary assembly of 3PS and 6PS at molar ratio of 4:1 in bulk solution. (a-c) A set of AFM images at different magnifications of the DNA arrays. (d-f) Inverse FFT images of (a-c) reconstructed by the circled diffraction spots in corresponding FFT patterns. Red lines in (d) indicate the boundaries of poly-grained crystals. White circles in (c, f) highlight the absence of 3PS. Scale bar: 200 nm for the inset image in (d).

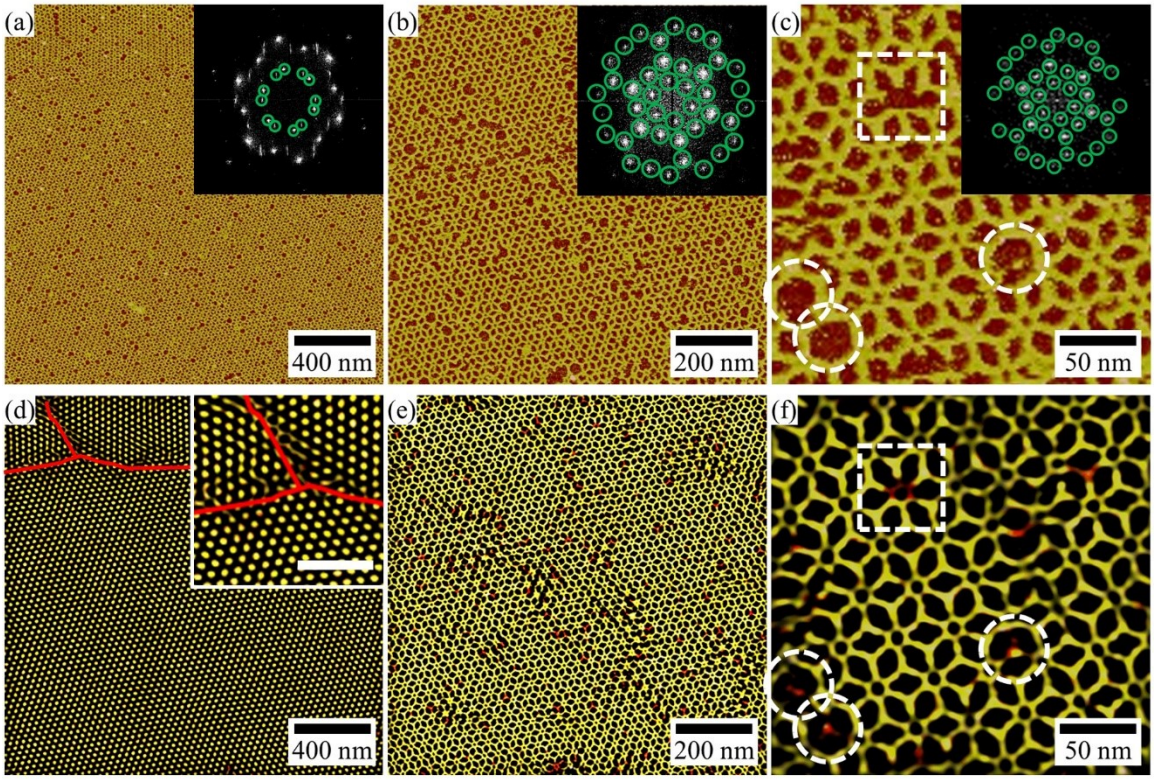


**Figure S7.** Binary assembly of 3PS and 6PS at molar ratio of 7:1 in bulk solution. (a-c) A set of AFM images at different magnifications of the DNA arrays. (d-f) Inverse FFT images of (a-c) reconstructed by the circled diffraction spots in corresponding FFT patterns. Red lines in (d) indicate the boundaries of poly-grained crystals. White circles in (c, f) highlight the absence of 3PS. White square in (c, f) highlights the absence of 6PS. Scale bar: 200 nm for the inset image in (d).

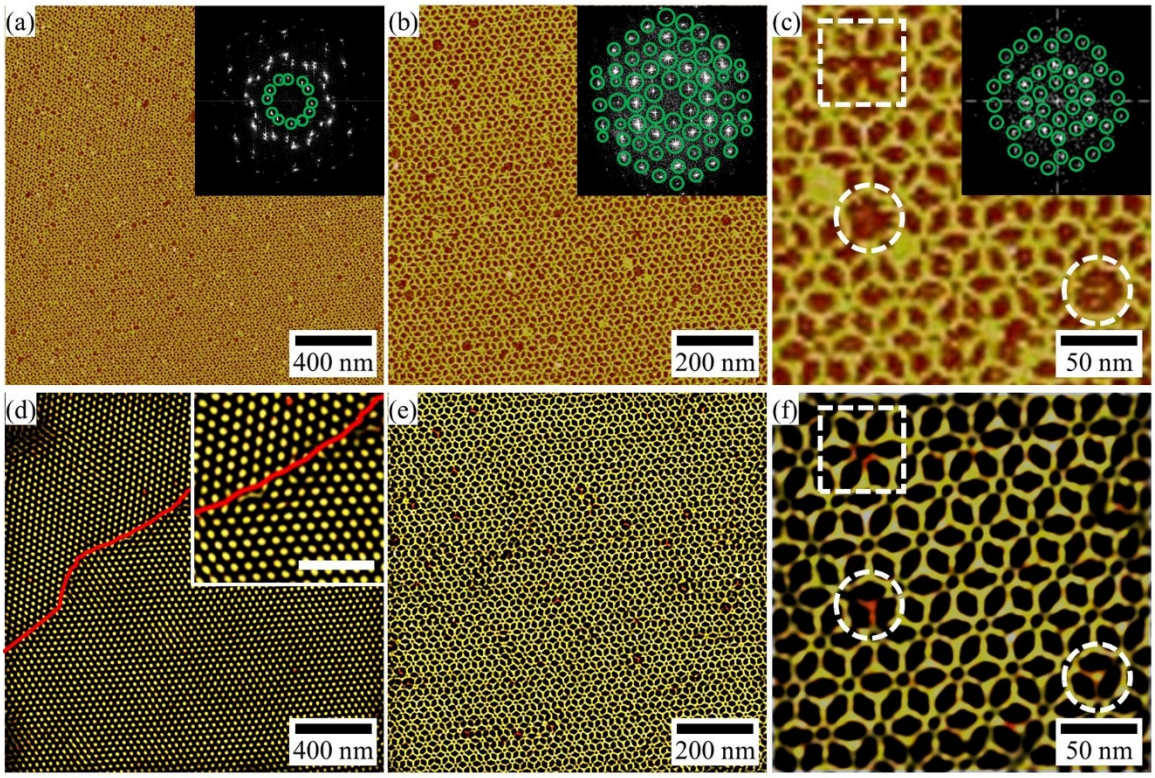


**Figure S8.** Binary assembly of 3PS and 6PS at molar ratio of 10:1 in bulk solution. (a-c) A set of AFM images at different magnifications of the DNA arrays. (d-f) Inverse FFT images of (a-c) reconstructed by the circled diffraction spots in corresponding FFT patterns. Red lines in (d) indicate the boundaries of poly-grained crystals. White circles in (c, f) highlight the absence of 3PS. White square in (c, f) highlights the absence of 6PS. Scale bar: 200 nm for the inset image in (d).

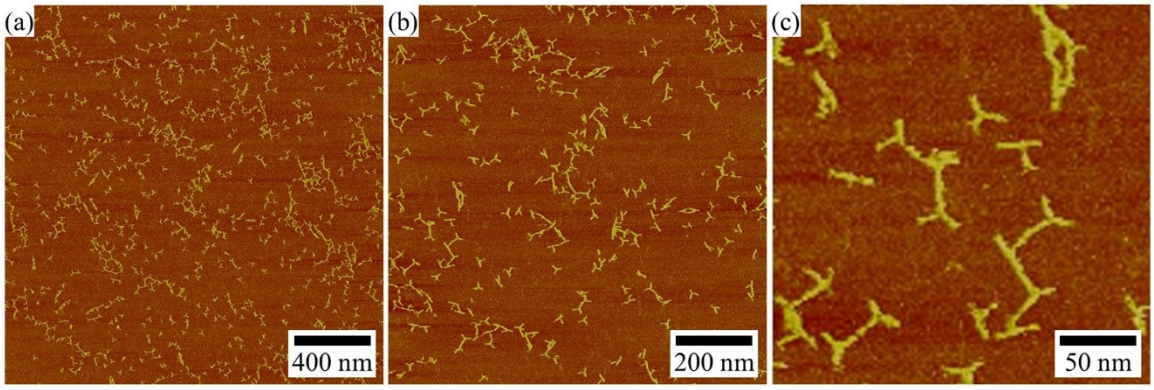


**Figure S9.** Incomplete arrays assembled from e3PS. (a-c) A set of AFM images at different magnifications of the DNA arrays.

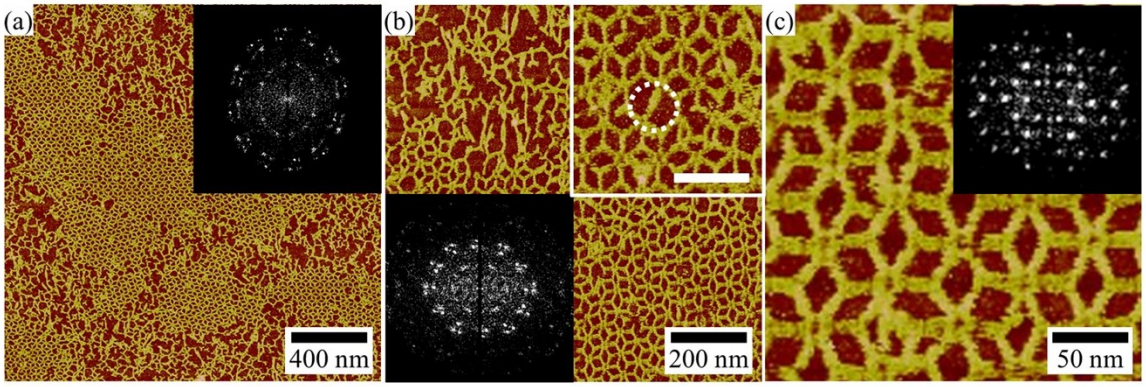


**Figure S10.** Binary assembly of e3PS and 6PS at molar ratio of 2:1 in bulk solution. (a-c) A set of AFM images at different magnifications of the DNA arrays. White circles in (b) highlight the deformed motifs. Scale bar: 100 nm for the inset images in (b).

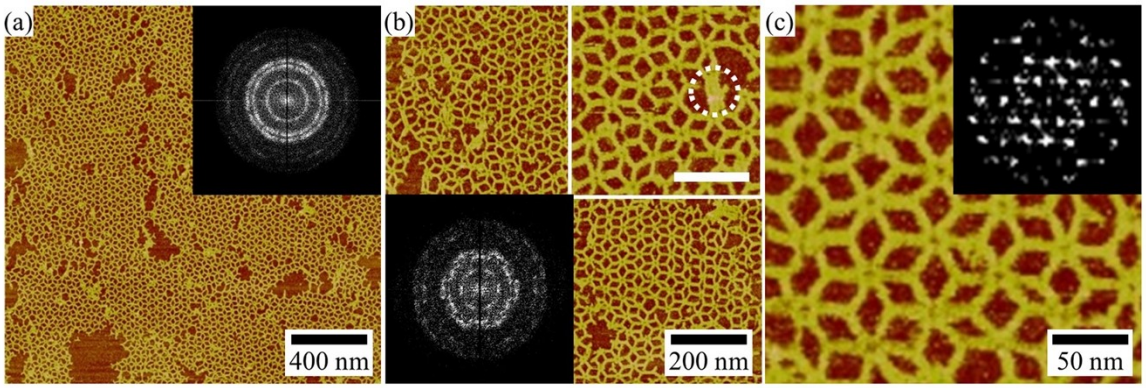


**Figure S11.** Binary assembly of e3PS and 6PS at molar ratio of 1:1 in bulk solution. (a-c) A set of AFM images at different magnifications of the DNA arrays. White circles in (b) highlight the deformed motifs. Scale bar: 100 nm for the inset images in (b).

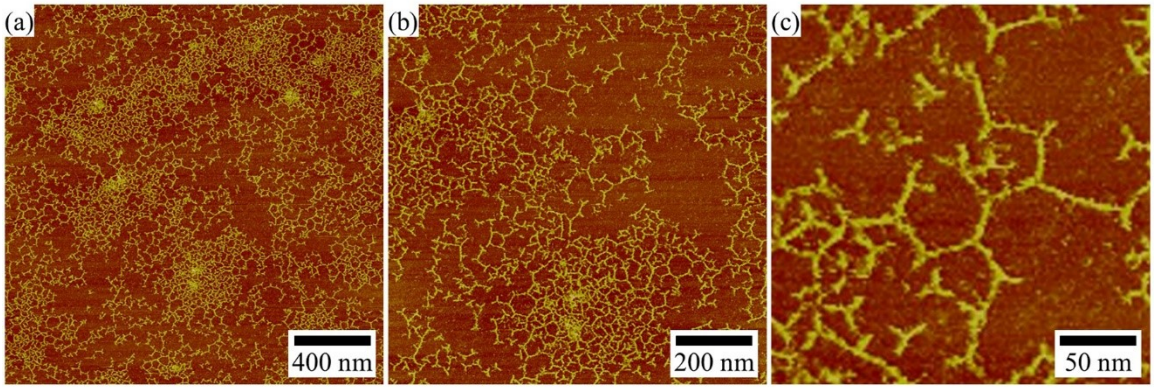


**Figure S12.** Incomplete arrays assembled from b3PS. (a-c) A set of AFM images at different magnifications of the DNA arrays.

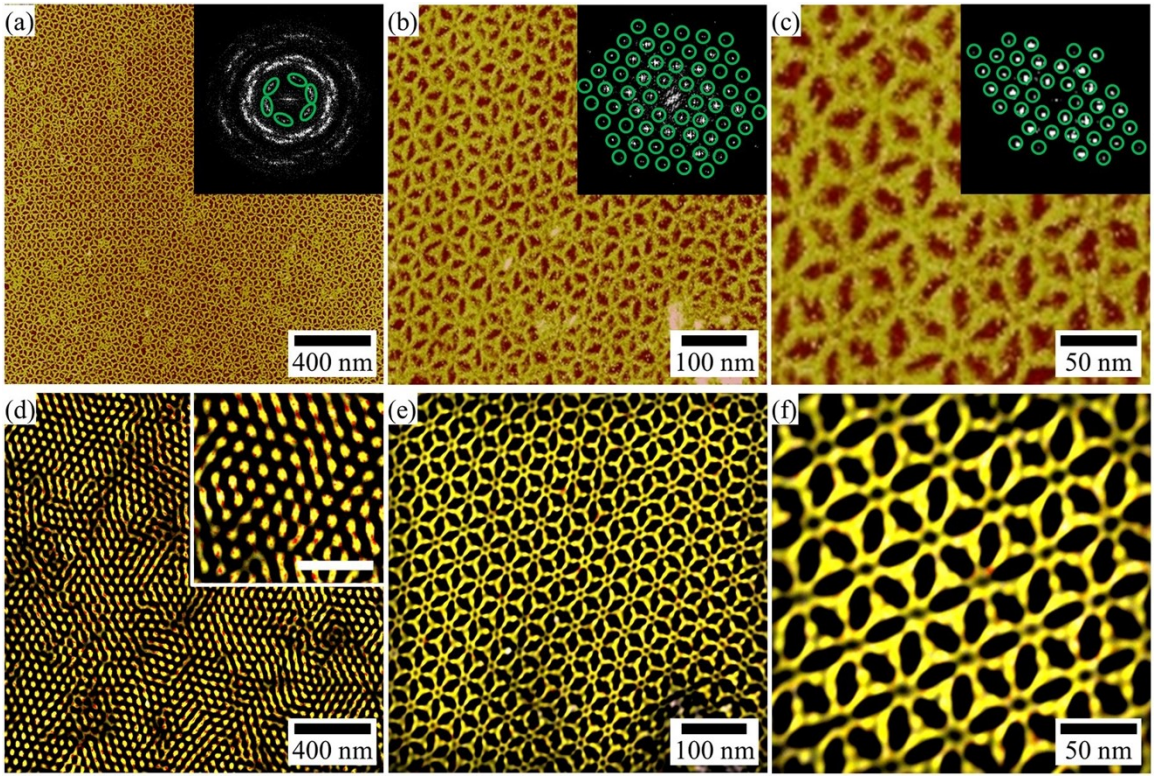


**Figure S13.** Binary assembly of b3PS and 6PS at molar ratio of 2:1 in bulk solution. (a-c) A set of AFM images at different magnifications of the DNA arrays. (d-f) Inverse FFT images of (a-c) reconstructed by the circled diffraction spots in corresponding FFT patterns. Scale bar: 200 nm for the inset image in (d).

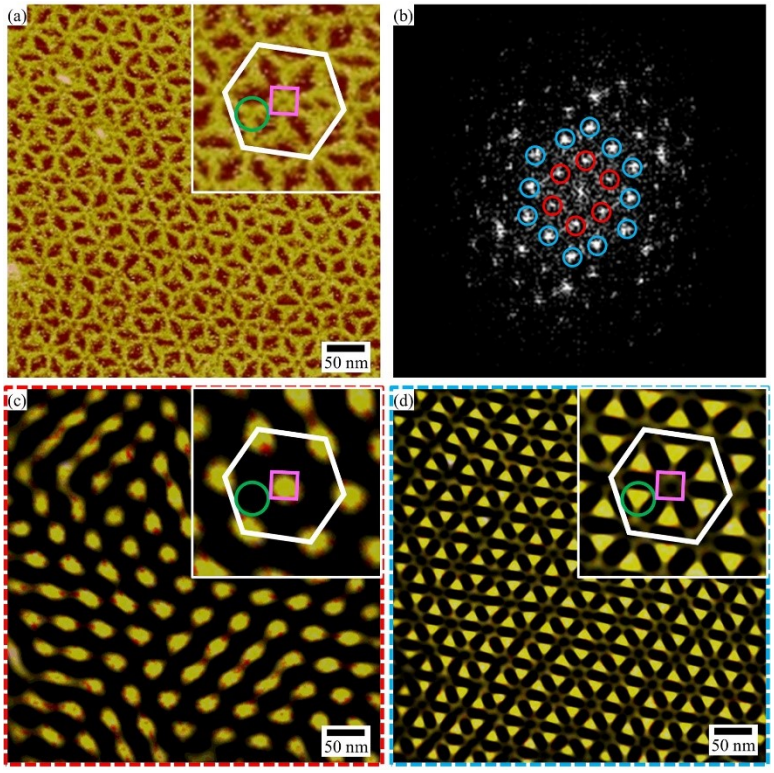

**Figure S14.** FFT analysis of the lattice assembled from b3PS and 6PS. (a) AFM image of the DNA array. (b) FFT pattern of (a). (c, d) Inverse FFT arrays reconstructed by diffraction spots in red, blue circles, respectively. Close-up views are shown at upper-right corner for (a, b, d). Six 6PS motifs stand at the vertices of the white hexagon. Inner 6PS is highlighted by the pink square. The b3PS is highlighted by the green circle.

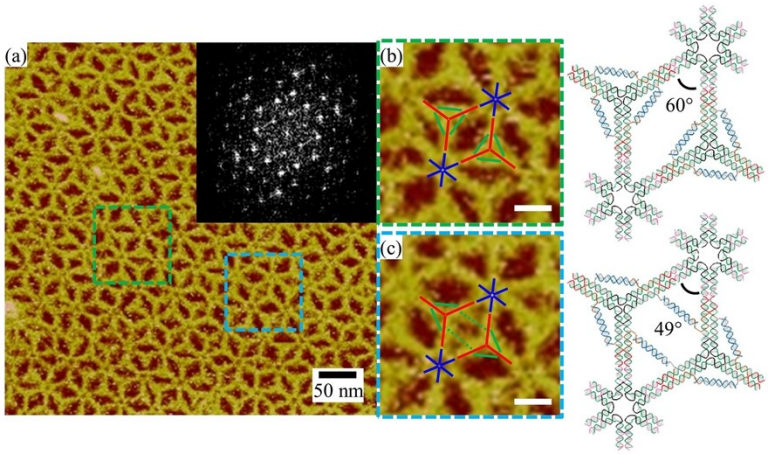


**Figure S15.** Bridge exchanges observed in the lattice assembled from b3PS and 6PS. (a) AFM image of the DNA array. Close-up views of b3PS of (b) regular state and (c) bridge-exchanged state in corresponding colored boxes from (a). Scale bar: 20 nm. Theoretical values are calculated for the acute angles.

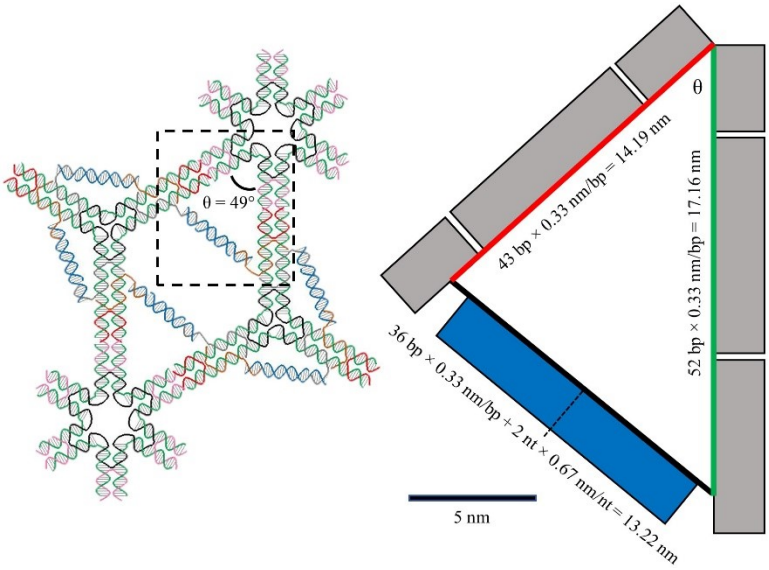


**Figure S16.** Calculation of the acute inter-branch angle θ. We assume the pitch of DNA duplexes is 0.33 nm/bp. The length of one base in ssDNA is 0.67 nm/nt. Calculated θ is 49°.