# Supporting Information for

# Isothermal assembly of DNA origami structures using denaturing agents

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## Materials and Methods

**DNA Origami Design.** The six-helix-bundle is composed of six double helices arranged in a hexameric fashion. The revised design of this 410 nm long version consists of 28 repetitive 42 bases long units, flanked by two end pieces (Figure S1). This allows the adjustment of the tube length by multiples of 14.3 nm, the length of 42 stacked bases. The sequences of the 186 staple oligonucleotides can be found in Appendix A.
The design of the rectangular origami structure was identical to that given in Ref. 1 of the Supplementary Information.

**DNA synthesis and sample preparation.**
**Origami rectangles.** Single-stranded M13mp18 scaffold DNA was purchased from New England Biolabs (Frankfurt am Main, Germany). Origami staple strands were purchased cartridge purified from biomers.net (Ulm, Germany) in water at 150 µM and used without further purification. 226 short strands for the formation of a rectangular origami structure[1] were mixed with M13mp18 (160 nM of each short strand, 1.6 nM M13mp18) giving a 100-fold excess of short strands.
**Six-helix bundles.** The recombinant M13 bacteriophage plasmid (p7560) was prepared replacing the BamHI-XbaI segment of the M13mp18 bacteriophage plasmid (p7249) by a 311 base sequence of the Lambda phage genome (sequence given in Appendix C) flanked by positions -25 to +25 of the middle of the XbaI cut site (T^CTAGA, or base 6258).
Recombinant M13 bacteriophage RF dsDNA was transformed into JM101 cells and grown overnight at 37 °C on an LB-agar plate. A single, well isolated plaque was used to inoculate 2 ml of 2xYT medium in a 14 ml sterile culture tube and agitated for 8 h at 37 °C. Bacterial cells were pelleted by centrifugation and the phage was recovered from the supernatant by polyethylene glycol fractionation (incubation on ice for 30 min with a final concentration of 4 % PEG8000, 0.5 M NaCl), followed by centrifugation. The phage was resuspended in 100 ml of 10 mM Tris-Cl (pH 8.5) and labeled "preinoculation phage." *Escherichia coli* JM109 cells were grown overnight in 3 ml of 2xYT medium at 37 °C. The 3 ml of JM109 culture was added to a 2 l flask containing 300 ml of 2xYT medium supplemented with $MgCl_2$ to 5 mM final concentration and incubated at 37 °C on a shaker at 300 rpm. When the bacterial culture reached $A_{600}$ = 0.5, 50 ml of the "preinoculation phage" stock was added. The infected culture was grown at 37 °C, shaking at 300 rpm for an additional 4 h. Phage was recovered as described above and resuspended in 3 ml of 10 mM Tris-Cl (pH 8.5) and labeled "inoculation phage." Titer of "inoculation phage" was measured by plating out serial dilutions using saturated JM109 culture and LB-top agar plates (35). Titer of JM109 cells at $A_{600}$ = 0.5 was measured by plating out serial dilutions on LB agar plates. For nanomolar scale production of phage, twelve 2 l flasks each containing 300 ml of 2xYT medium supplemented with 5 mM $MgCl_2$, were inoculated overnight with 3 ml of JM109 culture and incubated at 37 °C shaking at 300 rpm. When density reached $A_{600}$ = 0.5, each flask was infected with "inoculation phage" at an MOI (multiplicity of infection) = 1. Phage was harvested as described and resuspended in 0.5 % of the original culture volume in 10 mM Tris-Cl (pH 8.5). Single-stranded DNA was isolated from phage by alkaline/detergent denaturation as follows. Two

volumes of lysis buffer (0.2 M NaOH / 1 % SDS) were added to the resuspended phage, followed by 1.5 volumes of neutralization buffer [3 M KOAc (pH 5.5)]. Lysed phage was centrifuged for 10 min at 16000 × g. The supernatant was combined with one volume of 200 proof ethanol and centrifuged for 10 min at 16000 × g. Pelleted ssDNA was washed twice with 75 % ethanol, centrifuged, and resuspended in 5 % of the original culture volume in 10 mM Tris-Cl (pH 8.5). The concentration of the recovered ssDNA was estimated on an UV/visible spectrophotometer (Beckman Coulter) by using an extinction coefficient of 37.5 mg/ml for $A_{260} = 1$.
Six-helix bundle staple oligonucleotides were ordered from Invitrogen (Carlsbad, CA).

**Structure formation by thermal annealing.**
**Origami Rectangles.** For thermal annealing the mixture of short strands and M13mp18 DNA was brought up to a 100 µl volume using 1x Tris-Acetate-EDTA (TAE) buffer with 12.5 mM magnesium acetate (pH = 8.3). Annealing from 95 °C to 20 °C was done in a PCR machine (Eppendorf, Hamburg, Germany) at a rate of 1 °C/min in 0.1 °C steps.
**Six-helix bundles.** A volume of 20 µl containing 10 nM p7560 scaffold, 100 nM of each staple oligonucleotide, in folding buffer (50 mM HEPES, 30 mM $MgCl_2$, 50 mM NaCl, pH 7.5) was placed in a PCR tube in a Thermocycler (MJ Research, MA). The annealing protocol consisted of heating to 80 °C for 5 min and cooling down to 20 °C at 1 °C / 2 min in 1 °C steps.

**Structure formation by formamide denaturation.**
**Origami Rectangles.** The mixture of short strands and M13mp18 DNA was brought up to a 300 µl volume using 1x TAE buffer with 12.5 mM magnesium acetate (pH = 8.3) and 85 % formamide. The solution was placed in a microdialyzer (Spectra/Por® Float-A-Lyzer®, MWCO 3500, Spectrum Laboratories, CA, USA) and the dialyzer was fitted in a plastic tube containing 1x TAE/$Mg^{2+}$ buffer and 85 % formamide. This surrounding buffer solution was then gradually diluted with folding buffer solution containing no formamide by using a peristaltic pump (Reglo Analog MS 5-6, Ismatec, Switzerland) at a constant flow rate of 0.2 ml/min. After several hours, the DNA was recovered and imaged by AFM as described below. It was found that origami rectangles only require short annealing over 1h.
**Six-helix bundles.** A volume of 50 µl containing 4 nM p7560 scaffold, 40 nM of each staple oligonucleotide, folding buffer, and 85 % formamide was placed in a microdialyzer (QuixSep, TX) covered with a dialysis membrane (MWCO 3500, Spectra/Por® Spectrum Laboratories, CA). The microdialyzer was placed for 14 h in folding buffer containing 44 % formamide, then for 4 h in 22 % formamide and then further 4 h in 11 % formamide. The experimental setup is depicted in Scheme 1. The DNA was recovered by punching a pipette tip through the membrane. The samples were ready to image.

**Structure formation by urea denaturation.**
In addition to isothermal formamide denaturation, the origami rectangle structure was also formed by urea denaturation. The experimental procedures were equivalent to the formamide experiment, but instead of using 85 % formamide, we used 8 M and 16 M urea in the 1x TAE/$Mg^{2+}$ folding buffer. Results are shown in Figure S3.

**AFM imaging.** Samples were imaged in tapping mode using a Multimode AFM with Nanoscope IIIa controller and E-scanner (Veeco Instruments, Santa Barbara, USA). Imaging was performed in TAE/$Mg^{2+}$ buffer solution with NP-S oxide-sharpened silicon nitride cantilevers (Veeco Probes, Camarillo, USA) using resonance frequencies between 7-9 kHz of the narrow 100 µm, 0.38 N/m force constant cantilever.

After self-assembly of the origami structure ≈ 20 µl of TAE/$Mg^{2+}$ buffer solution was dropped onto a freshly cleaved mica surface (Plano, Dresden, Germany) glued to a metal puck (Plano). After 30 sec. the mica surface was dried using a gentle stream of $N_2$ and ≈ 5 µl of the origami solution was dropped onto the mica surface. After another 30 sec. ≈ 30 µl of additional buffer solution was added to the sample. After engage, imaging parameters were optimized for best image quality while maintaining the highest possible setpoint to minimize damage to the samples. Images were post processed by subtracting a 1st order polynomial from each scan line. Drive amplitudes were approximately 0.45 V, integral gains approximately 0.15, proportional gains approximately 0.3.

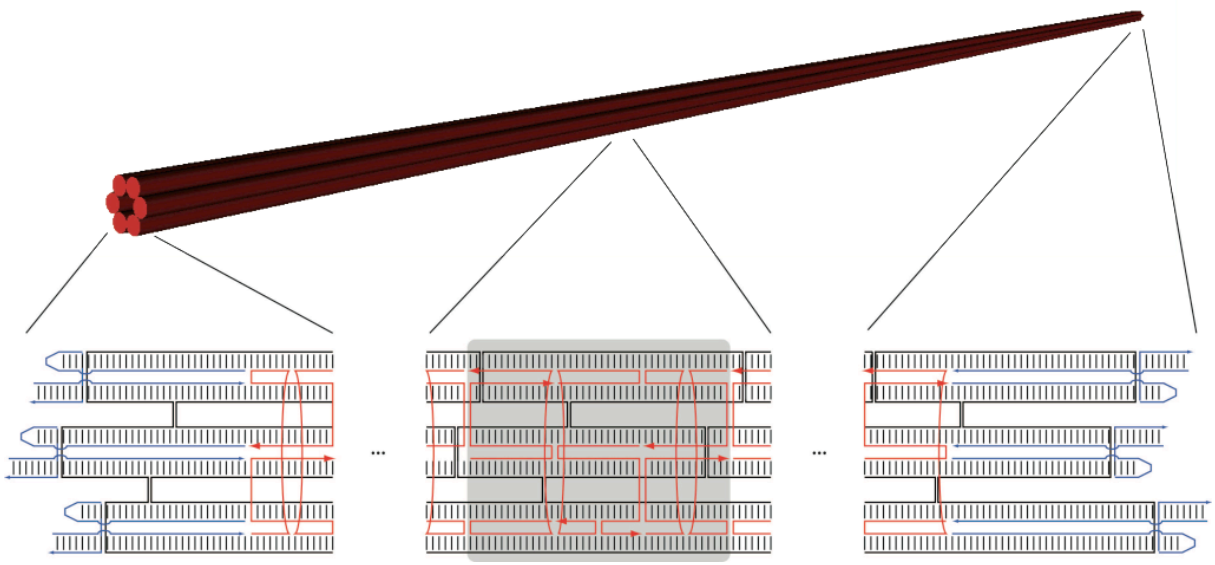
**TEM imaging.** After annealing, the DNA nanotubes were diluted to 1 nM in TBE. A 3 µl drop was placed on a Formvar®/carbon coated TEM-grid (400 Mesh 3.05 mm Copper, SPI Supplies, West Chester, PA). The drop was dabbed off with a filter paper after 20 s followed by rinsing with $H_2O$. The grid was then dipped for 20 s in a 50 µl drop of a negative staining solution, dabbed of and dried in the air. The negative staining solution was prepared directly before use as follows: 5 ml water was brought to boiling and 0.7 % uranyl formate (Pfaltz & Bauer, Waterbury, CT) was added. After 5 min of stirring and cooling to room temperature, 5 µl of 5 mM NaOH was added. The solution was filtered through a 0.22 µm syringe filter and ready to use. The tubes were imaged using a Tecnai™ G2 Spirit BioTWIN transmission electron microscope (FEI, Hillsboro, OR).

**Gel electrophoresis.**
**Origami rectangles.** For gel electrophoresis experiments 0.75 % Agarose (A9539, Sigma Aldrich, MO, USA) in 1x TAE buffer was heated to boiling and cooled to 50 °C and filled into the gel cask. Each gel lane was filled with 10 µl of 1.6 pM M13mp18 scaffold and thermal annealed origami structures and 10 µl of 0.5 pM formamide annealed origami structures. After running, the gel was stained with SYBR® Gold Nucleic Acid Gel Stain (Invitrogen, Carlsbad, CA, USA) and imaged with a custom made gel documentation system.
**Six-helix bundles.** 2% Agarose (Invitrogen, Carlsbad, CA) in TBE buffer was heated to boiling and cooled down to 50 °C. 11 mM $MgCl_2$ and 0.5 µg/ml Ethidium Bromide (Fisher Scientific, Pittsburgh, PA) were added to the liquid before filling it in the gel cask. Each gel lane was filled with approx. 100 fM of scaffold strand or folded DNA origami structure after the folding procedure. The gel was imaged with a AlphaImager© (Alpha Inotech, San Leandro, CA).

## References

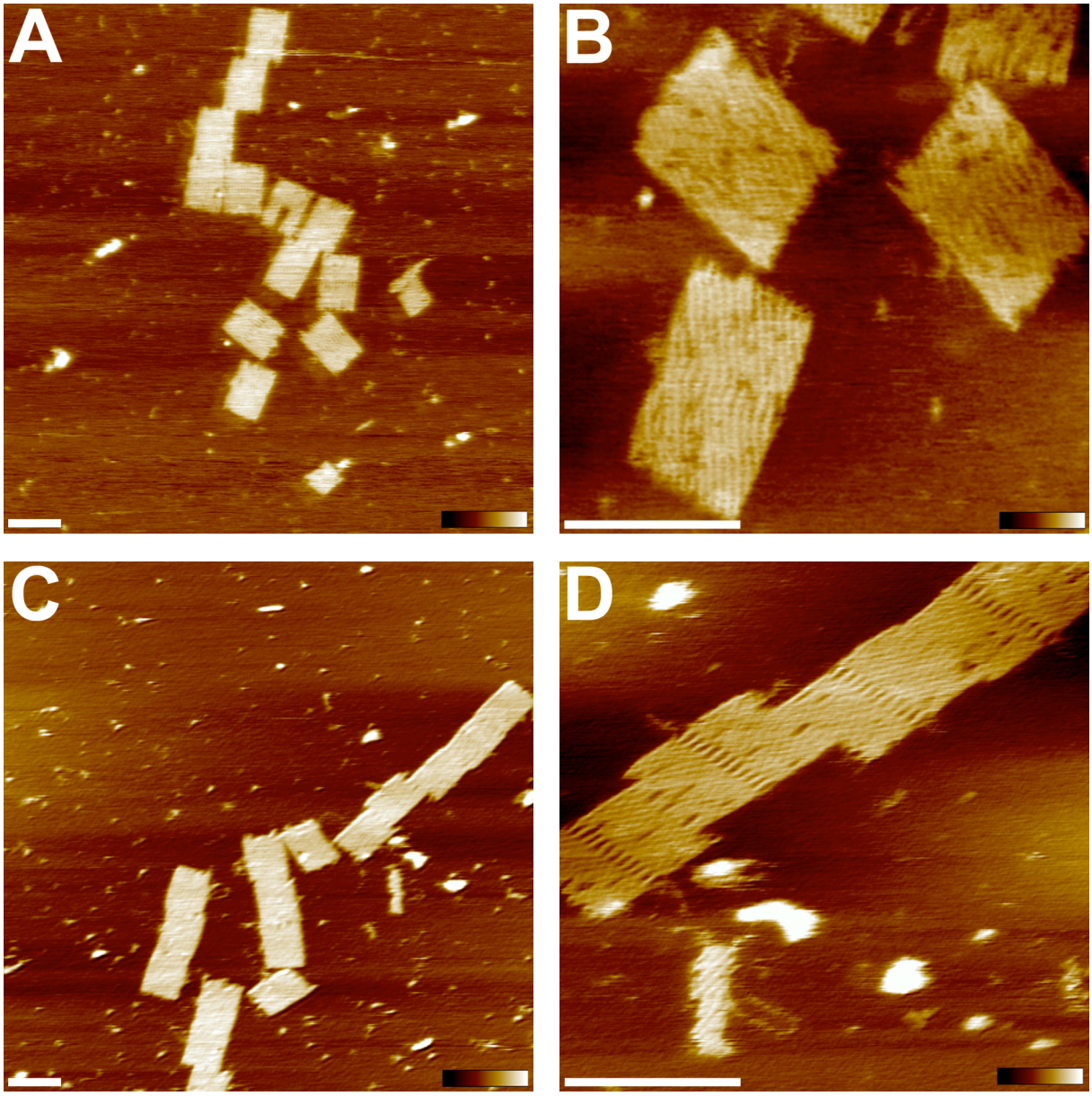
1. Rothemund, P. W. K. *Nature* **2006,** 440, (7082), 297-302.

**Figure S1**



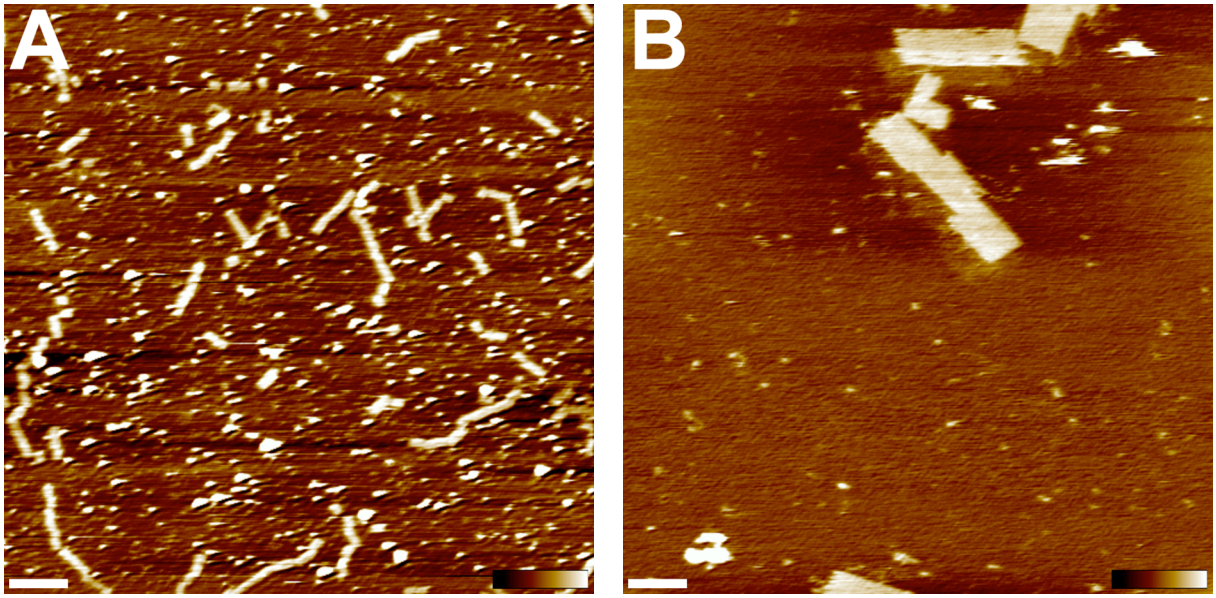
DNA tube design. Each cylinder in the upper image represents a double helix. One strand of each double helix consists of struts of the p7560 scaffold strand (black line) while the other strand consists of struts of the staple oligonucleotides (red and blue lines). The highlighted area depicts the repeated 42 base long unit is repeated 28 times in between the two end parts.

**Figure S2:**



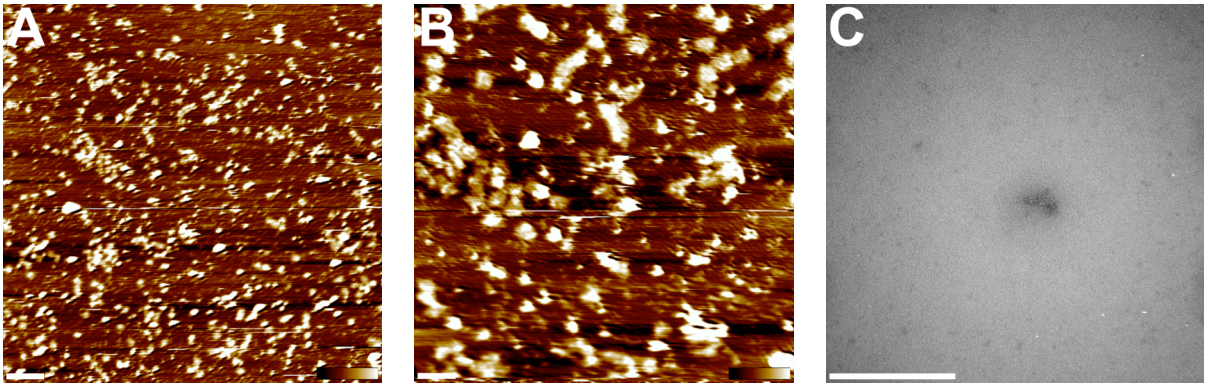

Rectangular DNA origami structures imaged in tapping mode under TAE/$Mg^{2+}$ buffer. The origami stamp structures in **A** and **B** were obtained by thermal annealing from 95 °C to 20 °C. The structures in **C** and **D** were prepared using the isothermal assembly method with formamide. **B** and **D** are zoom-in images of **A** and **C** respectively. Scale bars are 100 nm, height scale is 5 nm.

**Figure S3**



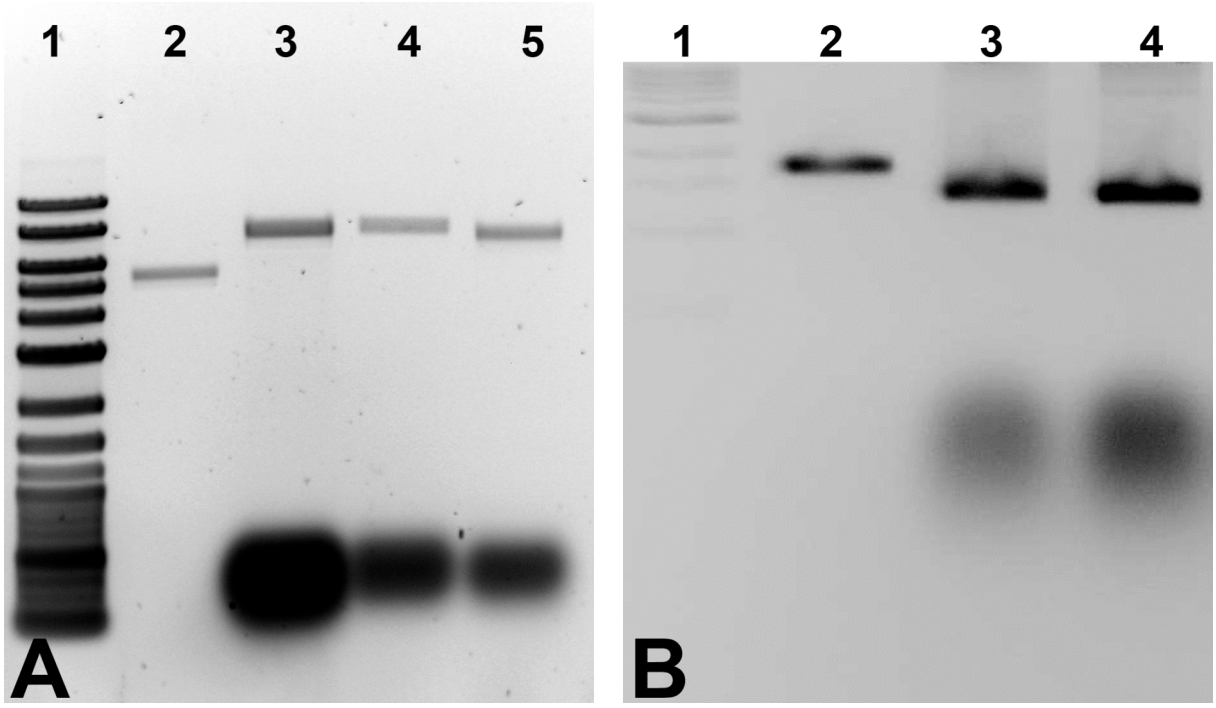

DNA origami stamp structures imaged in tapping mode under TAE/$Mg^{2+}$ buffer. The structures were obtained using the isothermal annealing method with urea. Structures in **A** and **B** were prepared using 8 M and 16 M urea concentration in the folding buffer respectively. Scale bars are 500 nm in **A** and 100 nm in **B**, height scale is 5 nm. Note the lower concentration of rectangles used compared to the thermal annealing protocol.

**Figure S4**




**A, B:** Origami rectangle solution imaged in tapping mode under TAE/$Mg^{2+}$ buffer. The solution was kept at room temperature over night and then imaged without using temperature annealing or the isothermal assembly method. No origami structures have formed. Scale bars are 500 nm **(A)** and 100 nm **(B)**, height scale is 5 nm. Features are salt residues and randomly assembled DNA. **C:** TEM image of result of incubation of six-helix bundle solution at 30°C for 48h. The scale bar is 500 nm.

**Figure S5**




Agarose gel electrophoresis of **(A)** rectangular and **(B)** six-helix bundle origami structures. Lanes in **(A)** are **(1)** 2log DNA ladder, **(2)** M13mp18 scaffold, **(3)** origami structures formed by thermal annealing, **(4)** origami structures formed by isothermal formamide annealing over 14 hr, **(5)** origami structures formed by isothermal formamide annealing over 1 hr. Note that for formamide annealing appr. 1/3 of the origami concentration for the thermal annealing experiment was used which is in good agreement with the gel data.
Lanes in **(B)** are: 1 kb ladder (**1**), Scaffold DNA (**2**), tubes prepared by isothermal formamide assembly over 18 h (**3**) and tubes prepared by thermal annealing over 2 h (**4**).

**Appendix A (sequence of 7560)**

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**Appendix B (Staple Sequences for Six-helix bundles)**

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