Electronic Supplementary Information

A versatile approach for geometry-based self-assembly of DNA-protein hybrid nanostructures using histone-DNA interactions

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Table of contents

Materials and methods

Purification of Core Histone

The purification of calf thymus core histones (Sigma-Aldrich) employed the acid extraction method. Initially, 4% perchloric acid (Sigma-Aldrich) precipitated the core histones, left to settle overnight at 4 °C. Following this, the core histones underwent centrifugation at a maximum speed of 15,000 rpm for 1 hour at 4 °C. After carefully decanting the supernatant; the resulting pellet was subjected to two successive washes with 1 mL of 4% perchloric acid, each followed by centrifugation at 15,000 rpm for 5 minutes at 4 °C. The histones were then further purified through two washes with 0.2% HCl in acetone and two with 100% acetone, with six washes performed in this manner. Following air-drying at room temperature for 20 minutes, the pellet was resuspended in ultrapure water. The resulting suspension was transferred to a spin filter (Sartorius - VIVASPIN2 5000 MWCO) and subjected to seven washes with cold distilled water (centrifugation at 15,000 rpm, 1 hour, 4 °C) to remove excess salt and concentrate the core histones. UV–vis absorption spectra were subsequently measured using a spectrophotometer (U-3900, Hitachi).

Preparation of HD Nanoparticles

Various ratios of core histones to 50-mer single-stranded DNA (ssDNA) from IDT were prepared, ranging from 0:1 to 40:1, at a low ionic strength of 1 mM Tris base with a pH of 7.4. After determining the quenching cutoff ratio (QCR), the self-assembly of HD nanoparticles was carried out using 175-mer ssDNA at a concentration of 120 nM. All reaction mixtures underwent an incubation period of 10 minutes at 4 °C. The fluorescence spectra of Cyanine5 (Cy5)-labeled 50-mer ssDNA from IDT were then recorded using a spectrofluorometer (Fluoromax-4, Horiba Scientific).

The sequences utilized in the core histones/ssDNA complexes are as follows:

50-mer ssDNA: 5’/Cy5/TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT

175-mer ssDNA: 5′ TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTCTTTCTTTCTTCTCTTCTTCTTCTT 3′

185-mer ssDNA: 5′ TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT TTTTTTTTTTGCTTTAGCTTTAGCTTAGCTGCTTGCTTCTTGCTT 3′.

DNA Nanostructure

DNA origamis (Tilibit Nanosystems) were outfitted with 25-nucleotide handles, bearing the sequence: 5′ AAGAAGAAGAAGAGAAGAAAGAAAG 3′. Dilution of DNA origamis to a final concentration of 5–20 nM was accomplished using sample buffer (1 × 5 mM Tris-base, 1 mM ethylenediaminetetraacetic acid (EDTA), 5 mM NaCl, and 5 mM $MgCl_2$ dissolved in ultrapure water).

After the self-assembly of HD nanostructures, the reaction mixture (comprising DNA origami and HD nanoparticles) underwent incubation at 4 °C for 1 hour. A consistent ratio of 1:1–1:2 DNA origami/core histones was employed across all experiments.

Self-Assembly of 2TD Hybrid Nanostructures

DsDNA arms were synthesized with the following sequences:

Strand.1:

TCCTACGCGTGACCCCGCTAGGACGGCATCTATATTAATAAGCCCCCGTCACTGTTGGTTGTAGAGCCCAGG ACGGGTTGGCCAGATGTGCGACTATATCGCTTAGTGGCTCTTGGGCCGCGGTGCGTTACCTTG

Strand.2:

CAAGGTAACGCACCGCGGCCCAAGAGCCACTAAGCGATATAGTCGCACATCTGGCCAACCCGTCCTGGGC

TCTACAACCAACAGTGACGGGGGCTTATTATCCTACGCGTGACCCCGCTAGGACGGCATCTATAT

The dsDNA arms were annealed using an annealing buffer and a thermal cycler (Thermal Fisher Scientific). After the self-assembly of HD nanostructures and dsDNA arms, the reaction mixture (comprising dsDNA arms and HD nanoparticles) was incubated at 4 °C for 1 hour. A consistent 1:1–1:2 dsDNA arms ratio to core histones was maintained throughout all experiments.

Agarose Gel Electrophoresis

Agarose gels (2%) were prepared by dissolving 2 g of GoldBio agarose LE in 100 mL of 1× TAE buffer (Omega Bio-Tek). The running buffer utilized for the experiment was TAE buffer (1X), fortified with 6 mM $MgAc_2$ from Sigma-Aldrich. The gel was stained using an ethidium bromide solution at a concentration of 0.025 μg mL^{-1} to visualize the DNA. Before loading onto the gel, all samples were supplemented with 10 mM $MgAc_2$ and mixed with gel loading dye purple from New England Biolabs. For reference, a 5 μL aliquot of 1 kb DNA ladder (GoldBio) was utilized, ensuring accurate size determination. Subsequently, 10 μL of the samples at a concentration of 10 nM were carefully loaded into the wells. The gel electrophoresis procedure was carried out at a constant voltage of 90 V for 2 hours, followed by an adjustment to 20 V for 24 hours, specifically after loading the DNA-HD nanoparticles hybrid nanostructures. Finally, the gel images were acquired using the Vilber E-Box gel imaging system, ensuring precise documentation of the results.

AFM

A precise technique was employed for AFM imaging in liquid: 10 μL of the samples was gently deposited onto freshly cleaved mica surfaces within a liquid cell. Following a brief 10-minute incubation, the liquid cell was meticulously filled with 0.5 mL of 0.5X TBE buffer, enriched with 200 $MgAc_2$ and 40 mM $NiCl_2$. The AFM measurements were carried out using the Bruker Dimension Icon AFM in ScanAsyst mode, employing a SCANASYST-FLUID+ probe (Bruker). Cantilevers with a frequency range of 100–200 kHz and a spring constant of 0.7 N m^{-1} were

carefully chosen for the task. The images were collected with a scan size ranging from 0.5 × 0.5 µm to 2 × 2 µm, maintaining a resolution of 256 × 256 pixels. To ensure precision, all images were processed using Gwyddion software.

A specific scanning approach was adopted for the imaging of dsDNA arms: a low scanning force of 0.5 pN and a fast-scanning rate of 2 HZ were meticulously selected. Similarly, the liquid cell was prepared with 0.5 mL of 0.5X TBE buffer, enriched with 200 $MgAc_2$ and 100 mM $NiCl_2$, ensuring optimal conditions for the imaging process.

Negative staining TEM

Samples at a concentration of 120 nM were carefully prepared for the experiment. The reaction mixtures containing dsDNA arms and HD nanoparticles were precisely incubated at 4 °C for 1 hour, maintaining a ratio of 1:1–1:2 DNA origami/core histones. To enhance visualization, negative staining was employed, utilizing a solution of 2% uranyl acetate to generate clear and detailed images.

Supporting Figures

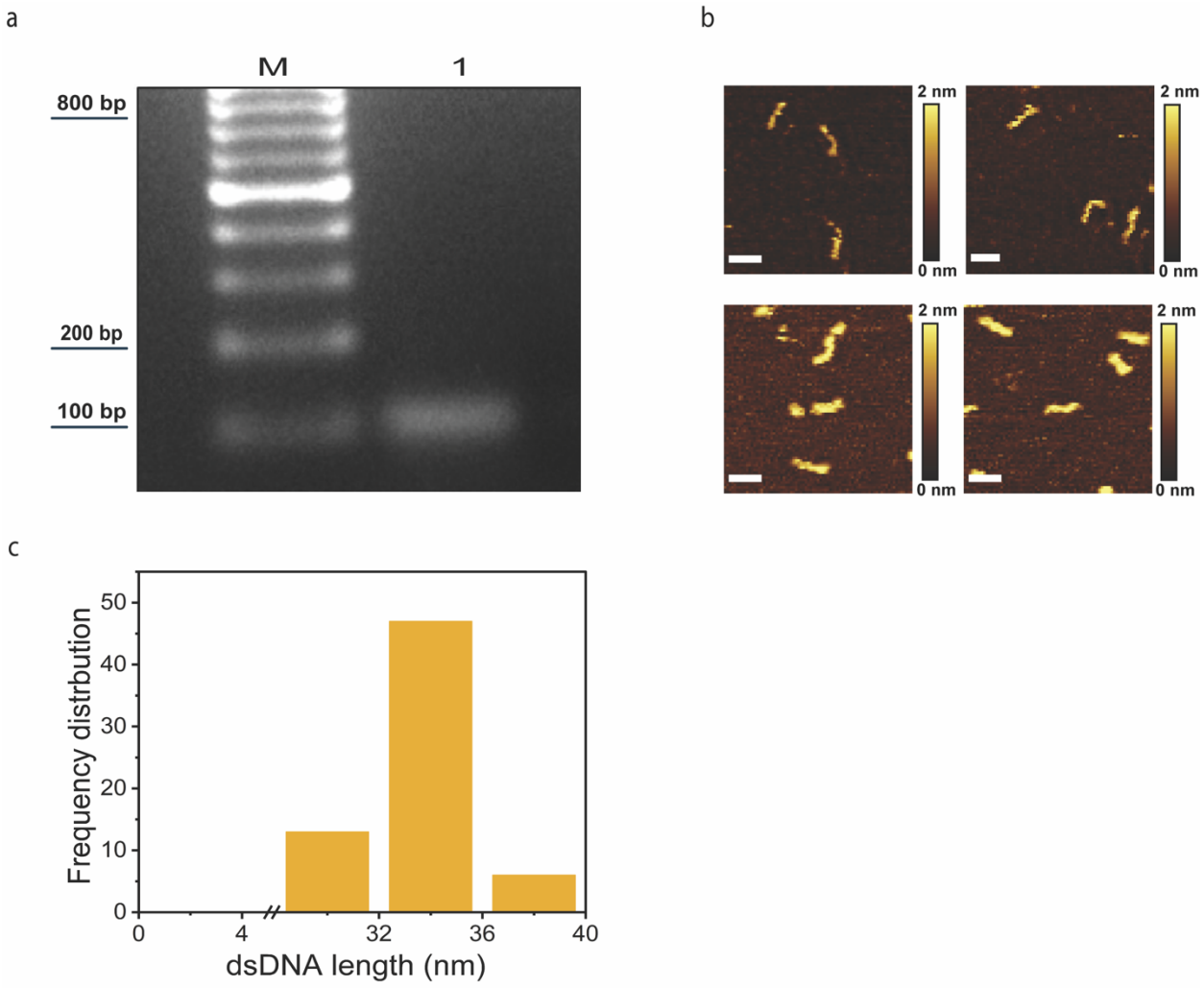

Fig.S1 Formation of dsDNA arms. The dsDNA arms, consisting of 100 base pairs (bp), are expected to have a length of approximately 34 nm. (a) Agarose gel electrophoresis (AGE) image of the linear dsDNA arms loaded in lane 1. (b) AFM images showing homogeneous dsDNA arms with the expected length of around 34 nm. Imaging conditions were optimized to avoid damaging the dsDNA arms (see Methods and Materials section). The scale bar shows a length of 30 nm. (c) Frequency distribution of the lengths of dsDNA arms as determined from AFM results.

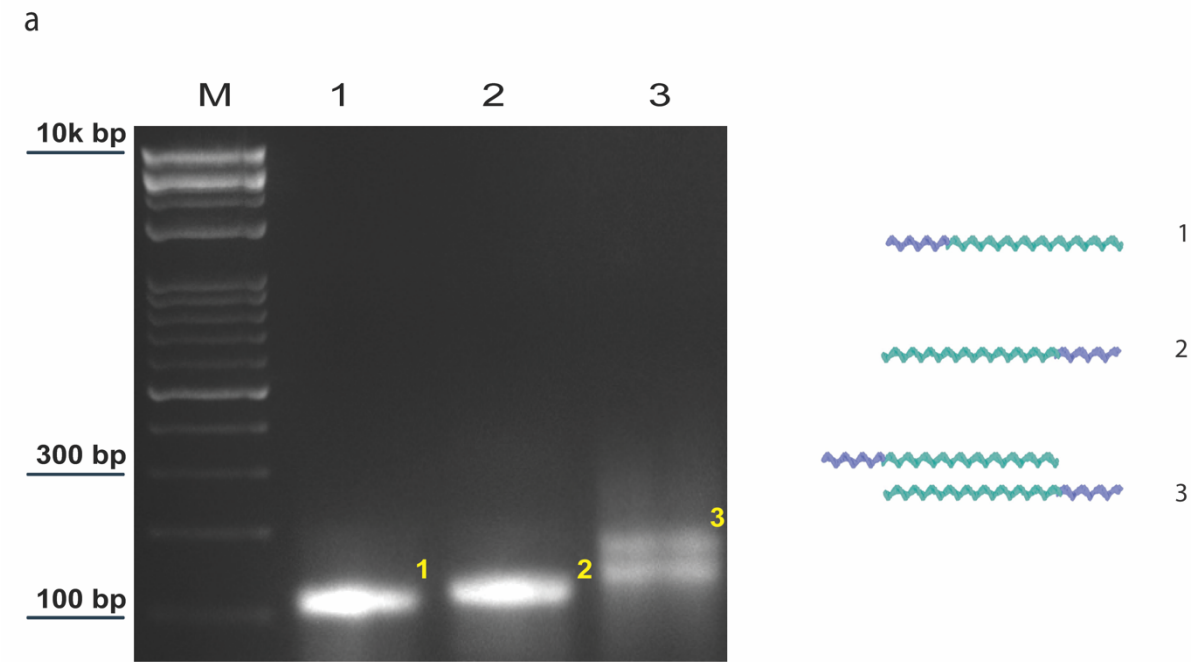

Fig.S2 Agarose gel electrophoresis (AGE) of dsDNA arms. Lanes 1 and 2 show ssDNA with sticky ends, and lane 3 shows the annealed dsDNA arms.

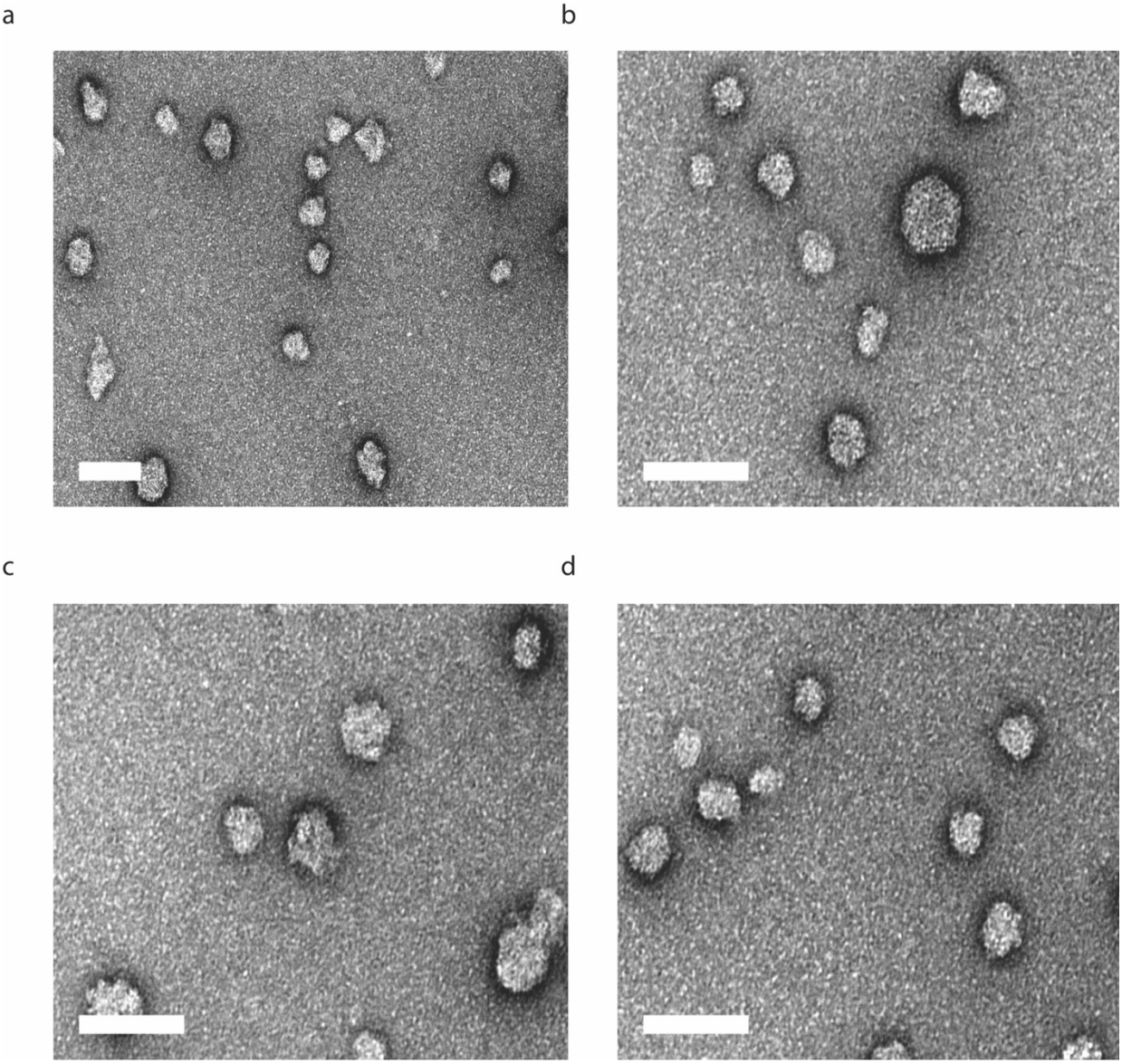

Fig.S3 Negative-stained TEM micrographs showing assembled tetrahedral DNA nanostructures (TDNs). (a-d) Widefield images reveal assembled 2TD formations along with variations arising from bond flexibility. All observed DNA nanostructures were under 300 nm in size. The scale bar shows a length of 100 nm.

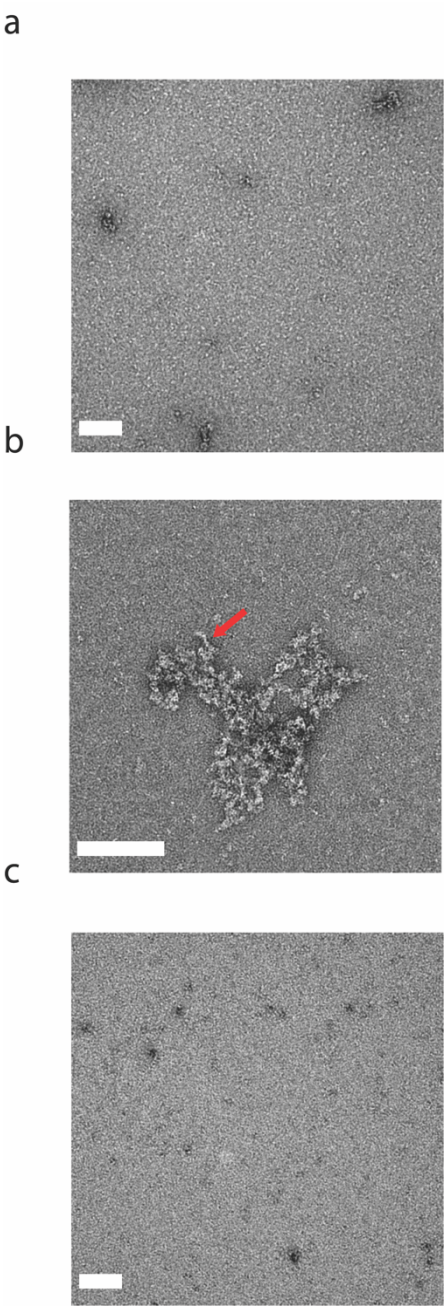

Fig.S4 Negative staining TEM images of dsDNA arms and ssDNA (i.e., control without histone protein). Three main types of samples were captured: (a) 100-bp dsDNA arms, (b) large helical DNA coiled to form random shapes with single helical strands indicated by the red arrow (diameter: 2 nm), and (c) flexible ssDNA forming small nanostructures. The scale bar shows a length of 100 nm.

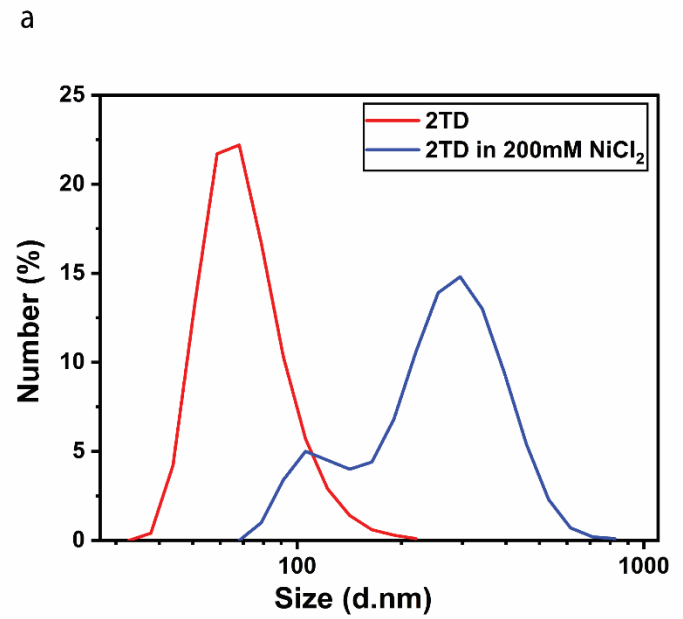

Fig. S5 Zetasizer analysis showing the size distribution of 2TD structures in media with varying ionic strengths.

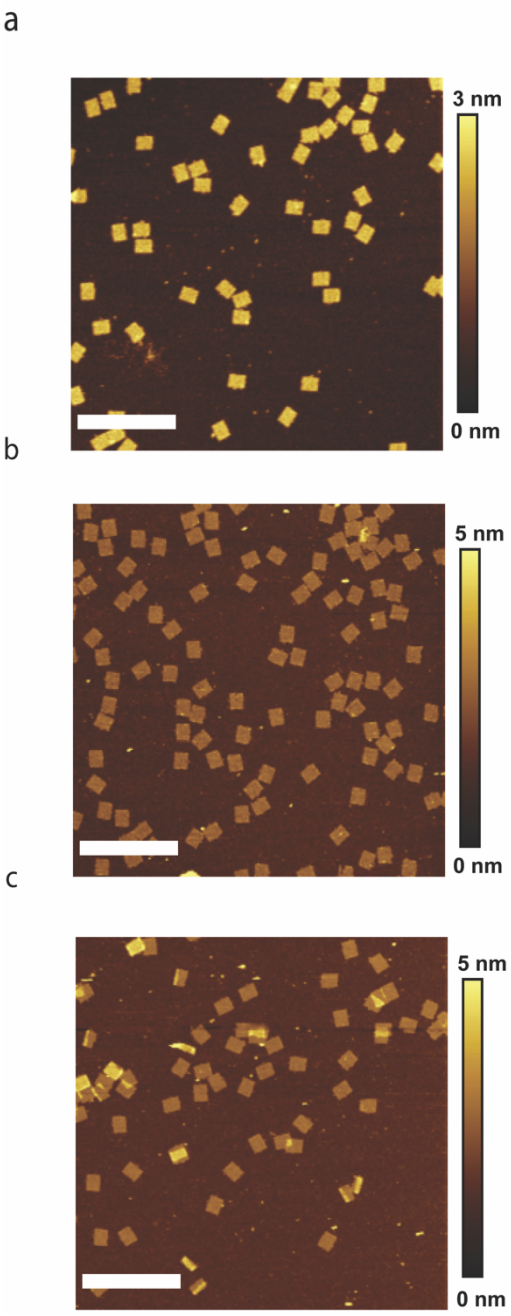

Fig.S6 Mono-layered squared DNA origami (SDO) was used as control samples to evaluate the assembly potential: (a) DNA origami alone, (b) DNA origami with ssDNA, and (c) DNA origami with histone. The scale bar shows a length of 500 nm