Molecular Conformations of Targets Captured by Model DNA Nanoarrays – Supporting information

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Experimental procedures

Materials.

Gold wire (99.99%, 1 mm diameter) was purchased from Scientific Instrument Services, Inc. 16-Mercaptohexadecanoic acid (MHDA), 11-Mercaptoundecanoic acid (MUDA) and 16-thiohexadecanol were purchased from Santa Cruz Biotechnology, Inc. $NiAc_2$, $MgAc_2$ were purchased from Sigma Aldrich. Tris-acetate-EDTA (TAE) and dithiothreitol (DTT) were used without further purification. Only ultrapure water (>18 MΩ·cm at 21°C) generated from a Barnstead Diamond Nanopure water purification system was used.

Preparation of the Thiolated DNA Probes.

The following synthetic oligonucleotides were used in this study:

Sf-Prime396 5'-ACCTTATGCGATTTTAAGAACTGG-3'

Sf-DraPrime+Tail 5'-CGTACTGACTGCTCACGAGGTAGC/iSpC3/TCTGAACTGTTTAAAGCATTTGAGGG-3'

SF-17 5'-GCTACCTCGTGAGCAGTCAGTACGTTTTT-3'/C11-SS

SF29-1k reverse primer 5'ACAGCTTGATACCGATAGTTGCG-3'

The undecyldisulfide-DNA (SF-17) was reduced by overnight incubation in an aqueous buffer solution containing a 1M DTT, and was purified using Illustra NAP-5 columns from GE Healthcare Life Sciences (Pennsylvania, USA). After reduction and purification, thiolated DNA probes were stored in TAE buffer, and the container was backfilled with nitrogen gas and stored at -20°C until use. DNA targets that have a 372 bp double-

stranded segment and a 24 *nt.* single stranded segment were prepared by conventional PCR amplification from the M13 bacteriophage genome, as described in a previous publication.[1]

The sequence of the dsDNA target is as follows:

5'-CGTACT GACTGC TCACGA GGTAGC TCTGAA CTGTTT AAAGCA TTTGAG GGGGAT TCAATG AATATT TATGAC GATTCC GCAGTA TTGGAC GCTATC CAGTCT AAACAT TTTACT ATTACC CCCTCT GGCAAA ACTTCT TTTGCA AAAGCC TCTCGC TATTTT GGTTTT TATCGT CGTCTG GTAAAC GAGGGT TATGAT AGTGTT GCTCTT ACTATG CCTCGT AATTCC TTTTGG CGTTAT GTATCT GCATTA GTTGAA TGTGGT ATTCCT AAATCT CAACTG ATGAAT CTTTCT ACCTGT AATAAT GTTGTT CCGTTA GTTCGT TTTATT AACGTA GATTTT TCTTCC CAACGT CCTGAC TGGTAT AATGAG CCAGTT CTTAAA ATCGCA TAAGGT-3'

Surface Monolayer Preparation.

A single-crystal gold bead substrate was prepared in house and used for all AFM experiments. The gold bead was prepared following an established protocol.[2] The gold bead substrate was cleaned by thorough rinsing with ultrapure water and organic solvents, followed by immersion in hot nitric acid or piranha solution (**3:1 sulfuric acid: hydrogen peroxide. CAUTION— piranha is highly corrosive and reacts violently with organics.).** The substrate was then rinsed with pure water and briefly annealed under a hydrogen flame, and then placed into 2mM thiol assembly solution at room temperature for overnight. Then the surface was rinsed with ethanol.

Atomic Force Microscopy and Nanografting.

An Agilent 5500 atomic force microscope was used for all experiments. SNL-10 (Bruker) probes with spring constants of approximately 0.2-0.4 N/m and a resonant frequency of approximately 16 kHz in liquid were used for all experiments. During imaging, topographical, amplitude, and phase channels were recorded. Typically equal volumes of TAE buffer containing 5-20nM of thiolated DNA probe and ethanol containing 20-400 μM of MUDA were added into a custom-made liquid cell for nanografting. Nanoshaving protocol was performed as previously described:[3] briefly, selected regions of the host self-assembled monolayer were removed by applying the lowest forces to the AFM probe using the PicoLith module of PicoView to achieve high-quality patterns into the host SAM. Generally, the force thresholds range between 100 and 200 nN.[3]

DNA surface hybridization

Prior to all hybridization experiments, the monolayer surfaces were repeatedly rinsed with an STAE buffer (saline Tris-acetate-EDTA: 200 mM NaCl, 40 mM Tris acetate, 5 mM EDTA, pH 8.3) to remove any Ni(II) ions on the surface. The substrates were then exposed to the target DNA (200nM) in a hybridization buffer containing 1.0 M NaCl, 1X TAE, and 1.0 mM SDS (sodium dodecyl sulfate) for a predetermined amount of time, after which the substrates were gently rinsed three times with STAE and placed under the imaging buffer (5mM Ni(II) in 0.1x TAE).

AFM image analysis

The experiments were repeated at least twice. 4 images were collected each time and around 120 DNA molecules were observed. DNA topographical heights and distributions were extracted using Gwyddion image analysis software (http://gwyddion.net/) and statistical analysis were performed using MATLAB script developed in the previous work.[1]

Additional data and AFM images

Before studying DNA interacting with patterned surfaces, we assessed the level of non-specific adsorption of DNA on ordered self-assembled monolayers on gold. After exposing an unpatterned 16-Mercaptohexadecanoic acid SAM on a single crystal Au(111) surface to a 1x TAE buffer solution containing 200 nM dsDNA targets, rinsing with the surface with the hybridization buffer, and imaging with AFM in the presence of Ni^{2+} that can immobilize DNA, we observed few if any DNA over many microns (Figure S1). The low level of non-specific adsorption is consistent with our previous study1 and suggests that the unpatterned host SAM is highly ordered and does not possess a significant level of surface defects that can trap DNA non-specifically. 1 Such a highly ordered SAM provides the opportunity to controllably introduce heterogeneities using surface patterning.

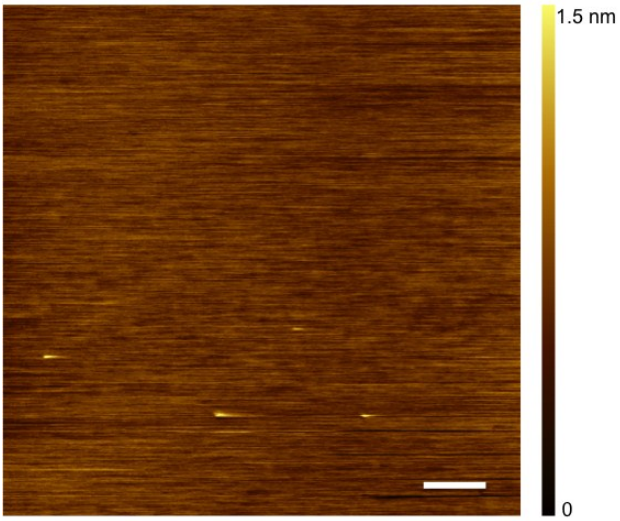

Figure S1 AFM image of non-specific adsorption of dsDNA on MHDA SAM on Au(111) surface. After exposing unpatterned MHDA SAM to a 1x TAE buffer solution containing 200 nM dsDNA targets, rinsing with the surface with the hybridization buffer, the SAM was imaged with AFM in the imaging buffer (5 mM Ni(II) in 0.1x TAE). The scale bar is 100 nm.

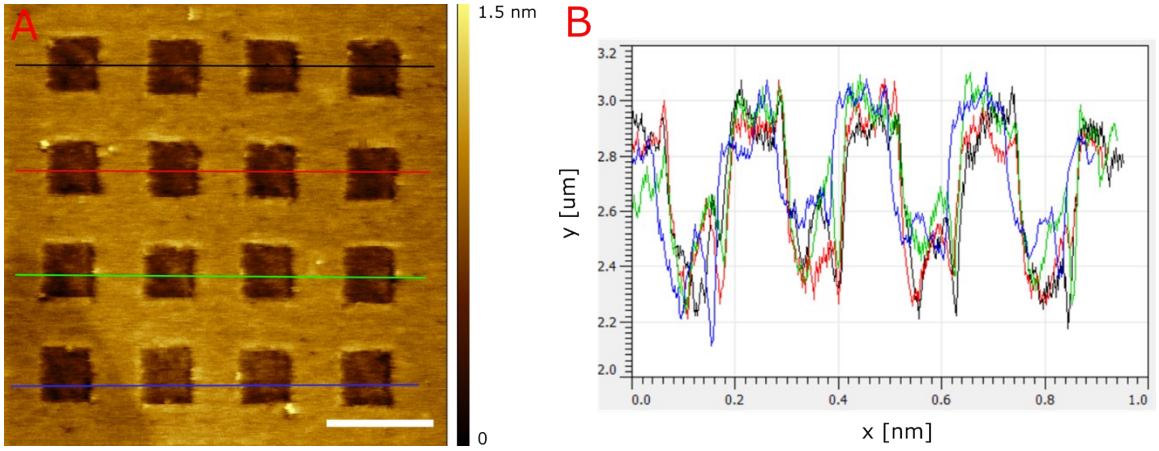

Figure S2 (a) Representative AFM image of nanografted MUDA squares in a MHDA host SAM. The scale bar is 200 nm. The cross-sectional profiles show that the squares are ~0.6 nm deep.

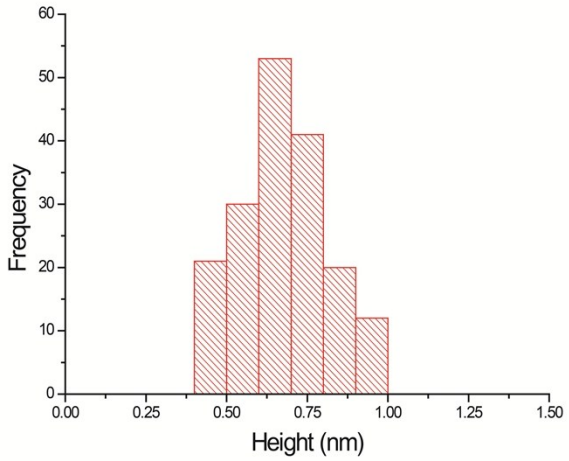

Figure S3 Histogram of the height distribution for ssDNA probe molecules in nanografted MUDA squares in MHDA host SAM. The DNA probes are 0.7±0.2 nm taller than the depressed regions.

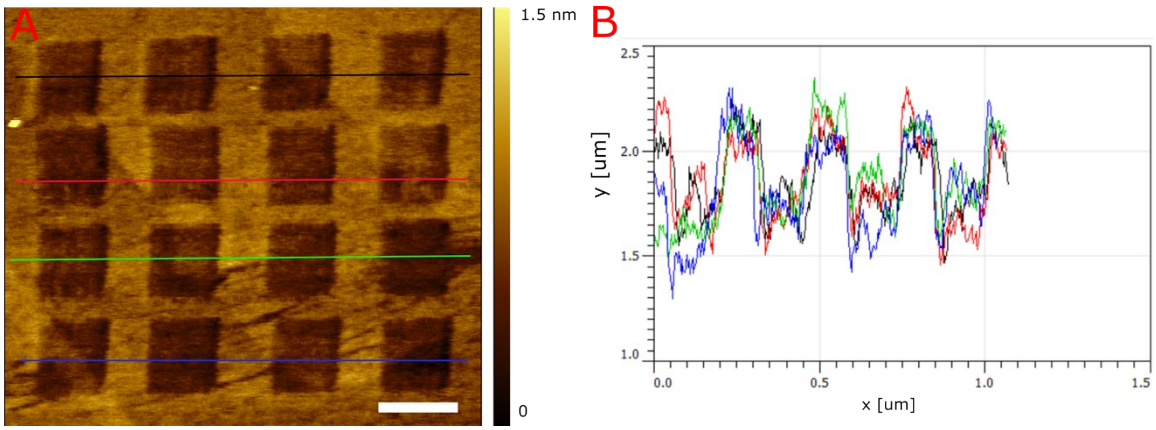

Figure S4 (A)AFM image of nanografted MUDA squares, which have no DNA probes, in MHDA host SAM after exposure to a 100 nM dsDNA target solution for 60 min. The lack of molecular-sized surface features inside the MUDA squares indicates that there is no nonspecific binding of DNA. The scale bar is 200 nm. (B) The cross-sectional profiles show that the squares are ~0.6 nm deep.

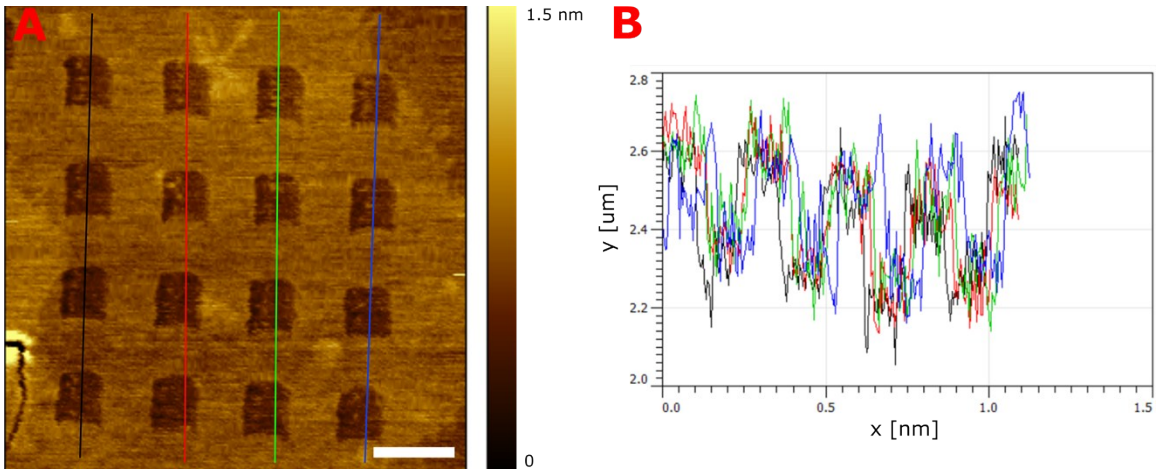

Figure S5 (A) Representative AFM image of nanografted MUDA squares with DNA probes. The image was acquired under 1:1 Ethanol/TAE buffer solution (the solution used for nanografting). (B) Cross-sectional profiles show that the squares are ~0.2-0.3 nm deep. The scale bar is 200 nm.

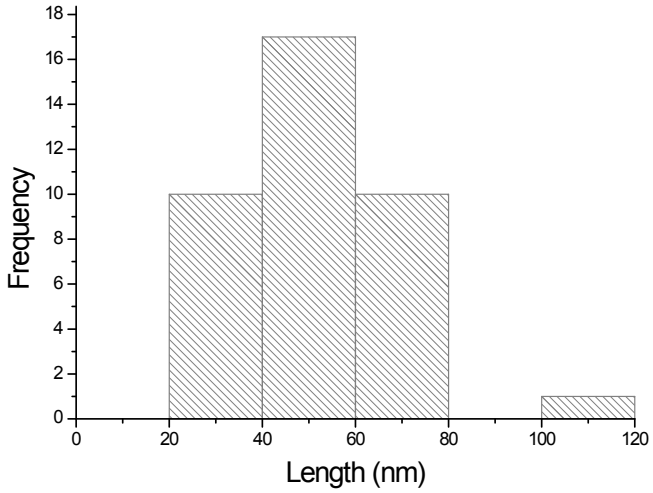

Figure S5 Histogram of the immobilized lengths of dsDNA on MUDA/MHD patterns. The average length, 50 nm, is much shorter than the full contour length of DNA, which is about 130 nm. It indicates that only a part of the molecule is pinned to the surface and the rest of the DNA is mobile and does not appear in AFM imaging, since the dsDNA cannot be immobilized on the MHD SAM, which is hydroxyl terminated.

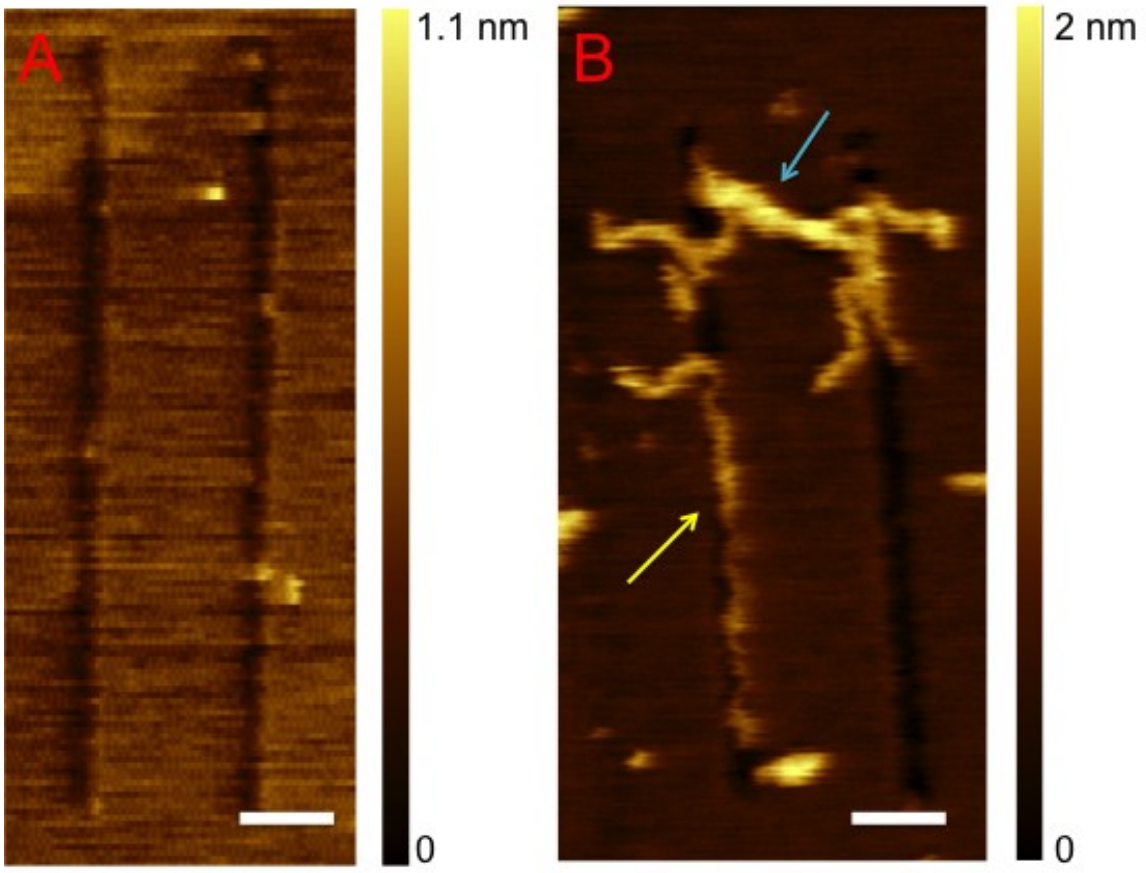

Figure S6 (A) Representative AFM image of nanografted MUDA channels with DNA probes, but without DNA hybridization. **(B)** AFM image of 1000 bp dsDNA aligned with MUDA/MHDA boundaries. The image was acquired under 5mM Ni(II) in 0.1x TAE buffer solution. The DNA duplex is notably longer, 320 nm. Therefore, while some of them are aligned along a trench (e.g., yellow arrow). Others may cross over to the neighboring channel (blue arrow). Scale bars are 100 nm.

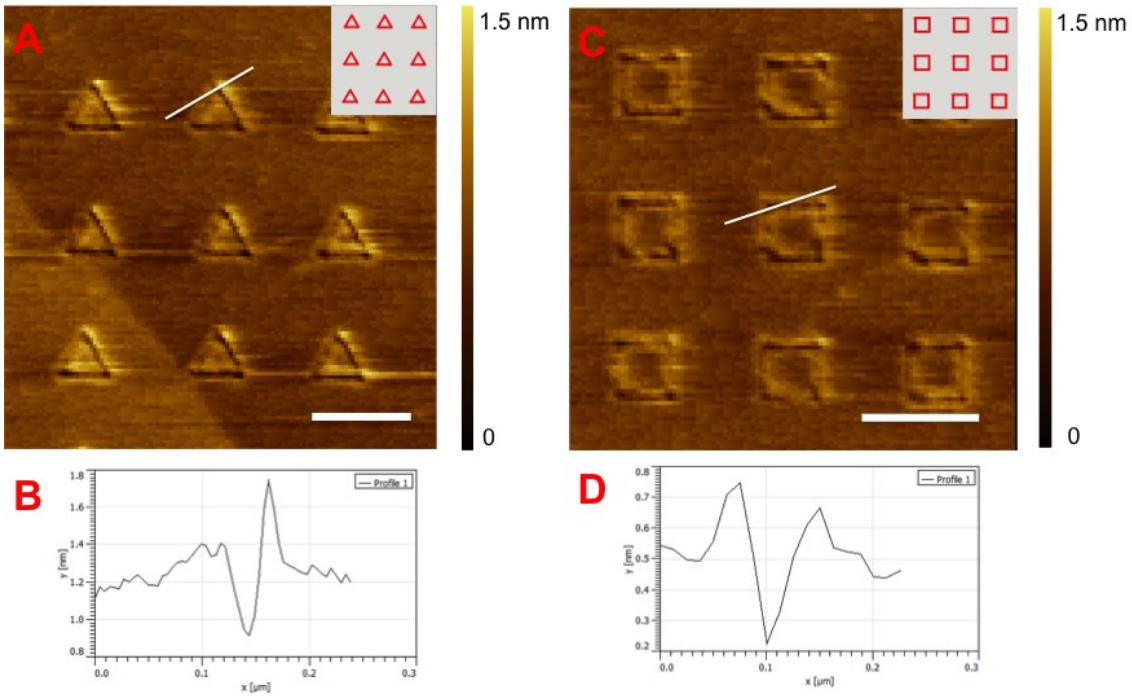

Figure S7 (A) and (C) AFM images of nanografted MUDA with DNA probe, but before dsDNA hybridization by rectangular and triangular frames, respectively. (B) and (D) are cross sectional profiles of (A) and (C), respectively. Images were acquired under a 0.1x TAE buffer solution that contained 5mM Ni(II). Insets are the designs of surface patterns. Some protrusions, 0.2-0.5 nm high, are observed at the edges. The scale bar is 200 nm.

The origin of the protrusions in Figure S7 is unclear. It is possible that DNA probe jamming occurred in confined spaces and the DNA portion of some DNA probes was forced out of the nanografted MUDA channel and pinned atop the MHDA. Another possibility is that deformation occurred in the MHDA SAM near the nanografted region. The MHDA molecules may be forced to assume a more vertical orientation and become taller. Regardless of the origin, the height of the protrusions is much lower than 2.0 nm, the height of the protrusions in Figure 4. Hence the features observed in Figure 4 correspond to captured dsDNA target molecules.

1. Abel Jr., G. R.; Josephs, E. A.; Luong, N.; Ye, T. *J. Am. Chem. Soc.* 2013, **17**, 6399.

2. Josephs, E. A.; Ye, T. *J. Am. Chem. Soc.* 2012, **24**, 10021.

3. Josephs, E. A.; Shao, J.; Ye, T. *Nanoscale* 2013, **10**, 4139.