Folding Single-Stranded DNA to Form the Smallest 3D DNA Triangular Prism

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Supporting Information

I. Materials

The DNA oligonucleotide of 198 nucleotides, designed using Uniquimer, was purchased from Integrated DNA Technologies, Inc. 100 base-pair (bp) DNA ladders were purchased from Fermentas International, Inc. Mung Bean nuclease was purchased from Epicentre. GelRed was purchased from Biotium Inc. All other reagents were purchased from Sigma-Aldrich.

II. Hybridization

The single-stranded DNA (198 nucleotides, shown in Table S1) was dissolved in 1×TAE/Mg^{2+} buffer (Tris-acetic acid 40 mM, pH 8.0, magnesium acetate 12.5 mM, and ethylene-diaminetetraacetic acid (EDTA) 1 mM) to reach the concentration of 0.5 μM and annealed in a water bath from 95 °C to room temperature for approximately 48 h. The synthesis of 5-stranded DNA triangular prism is in the similar way with an equimolar mixture of the component strands. The annealed DNA sample was analyzed by native 12% PAGE in 1×TAE/Mg^{2+} buffer under 4 °C.

Table S1. DNA sequence of 198 nucleotides

GACACTGCGAGGCTATTGTTCTGCTCATCAGGCCATAGTTGC
AGGTGTATGCCGTAAGATTCGTATATCAGTGTGTCGTAGATTG
AGCAGAACTCACAGTAACCTTACACCTGCATCTATAAGGCGT
ATCTTACGGCTGGTTACTGTGTATAGCCTCGCTCTGATATACGT
CGCCTTATAGTCTATGGCCTGTTCTAC

Sequences of 5-stranded DNA triangular prism:

Strand 1: ATACGTATCTTACGGCTGGTTACTGTGTATAGCCTCGCTCTGAT

Strand 2: GTGTATGCCGTAAGATTCGCCTTATAGTTGCAG

Strand 3: AGAACTCACAGTAACCTTACACCTGCATCTATGGCCTGTTGAGC

Strand 4: GCTATTGTTCTGCTCATTCTACGACACTGCGAG

Strand 5: AGGCGTCGTATATCAGTGTGTCGTAGATCAGGCCATAGTCTATA

III. Enzyme Digestion

To confirm the double-stranded nature of the triangle prism, enzyme digestion using Mung Bean nuclease was conducted. To ensure the enzyme activity and to determine the optimal conditions under which single-stranded DNA can be completely digested, the single-stranded DNA before annealing was digested by different amounts of Mung Bean nuclease. Two picomoles of DNA were added into 1× Mung Bean reaction buffer (300 mM sodium acetate (pH 4.6), 500 mM NaCl, 10 mM zinc acetate and 0.1% Triton X-100) with 0.5, 1.0 units of Mung Bean nucleaseand incubated at 37 °C for 1 hour. As the result shown in Figure S1, the sample with 1.0 unit of the enzyme was completely degraded. The mixture of the single-stranded DNA before and after annealing was analyzed by Mung Bean nuclease under the same enzymatic digestion. Two picomoles of DNA were digested by 1.0 units of enzyme in 15 μL of 1× Mung Bean reaction buffer at 37 °C for 1 hour. The digested products were analyzed by native 12% PAGE in 1×TAE/Mg^{2+} buffer.

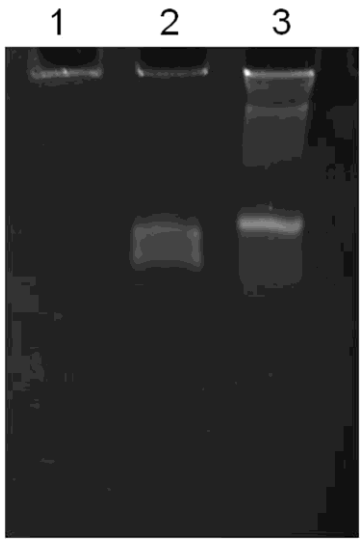

Figure S1. 12% PAGE results of the single-stranded DNA with different units of Mung Bean nuclease. Lane 1 was loaded with the mixture of DNA and 1.0 unit of Mung Bean nuclease; lane 2 was loaded with the mixture of DNA and 0.5 unit of Mung Bean nuclease; lane 3 was loaded with only DNA.

IV. AFM Imaging

Liquid phase:

A3 μL of DNA prism sample with a concentration of 20nM was deposited onto a freshly cleaved mica (SPI Supplies Division Structure Probe, Inc.) and left to adsorb for 2 min. We added additional Ni^{2+} (40 mM) to increase the binding effect of DNA-mica. Then, the mica was fixed into the liquid cell and buffer (1× TAE/Mg^{2+}, 150uL) was added into the liquid cell. After placed the cell, the images were obtained under semi-contact mode using DNP-S cantilevers (Veeco, Inc.).

Air phase:

A 3 μL of DNA prism sample with a concentration of 15 nM was deposited onto a freshly cleaved mica (SPI Supplies Division Structure Probe, Inc.) and left to adsorb for 3 min. The sample was scanned in air phase under semi-contact mode on AFM (NT-MDT, Inc.) with NSC15 cantilevers (MikroMasch, Inc.).

V. STM Imaging

All the STM experiments were carried out with constant current mode (bias voltage V= +3.0 V, tunneling current I = 0.02 nA.) at 77 K using a commercial ultra-high-vacuum low-temperature (UHV LT-STM) system (Omicron Nanotechnology). Under such extreme conditions, the disturbances occurred in ambient STM, e.g., dust contamination,

moist, thermal fluctuation or tip instability caused artifacts are greatly minimized. In recent years, several groups have demonstrated that UHV LT-STM can be used to verify the structures of biological systems.[S1-S3] It is worthwhile to compare STM with cryo-electron microscopy (cryo-EM) technique. The later one detects electron beam signal transmitted through a 3D object at a specific projection. Because of the low contrast of biological spices and the low-level electron does in order to minimize the radiation damage, the signal-to-noise ratio of the resulting images is very low, so that multiple copies of a structure must be averaged.[S4-S5] In contrast, STM reveals the topography of 3D objects adsorbed on a substrate with appreciable signal-to-noise ratio. As the objects frequently adsorb in different configuration or conformation, averaging STM data will result in distorted topography. So, we do not average the STM data.

The substrate used in our study was gold film coated on mica and cleaned by sputtering of Ar^+ and post annealing treatment. A 1 μL drop of DNA sample with a typical concentration of 20 nM was injected onto the Au (111) surface in N_2 atmosphere which was quickly pumped away afterwards. Small current (0.02 nA) and high bias voltage (+3.0 V) were applied for a stable scanning in order not to disturb the DNA nanostructures.

If the demineralization was too much for the DNA solution or the prepared sample was kept at room temperature for a long time (over three days, even in UHV), the morphology could be quite different for the sample, as shown in Figure S2. Large clusters with a size of ~8 nm and a height of ~1.5 nm were all over the surface (Figure S2a). A detailed observation could be found in zoom-in images Figure S2b and S2c. According to their dimension, we thought that these clusters should be an aggregation of DNA molecules and fragments. It could be caused by the lack of salts in the solution preventing the nanostructures from decompositionor the temperature influence resulting in the unwinding of DNA molecules, which was also observed in the AFM experiments.

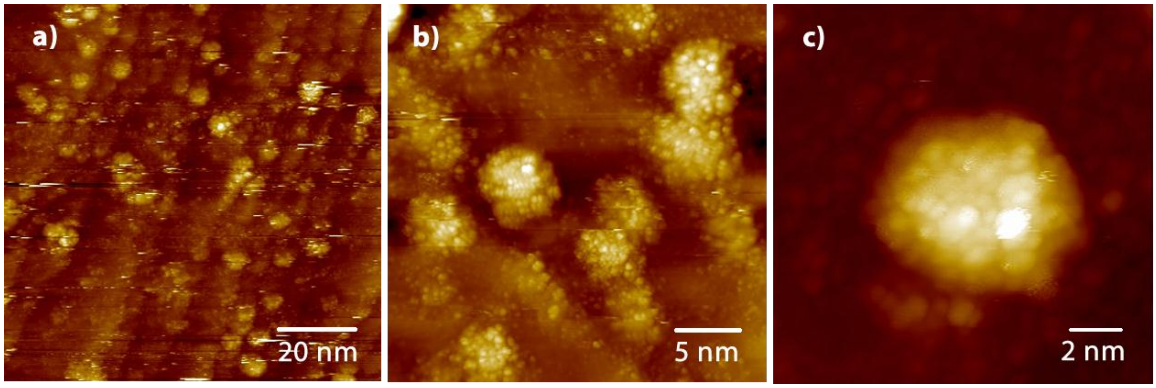

Figure S2. STM images of large clusters on Au (111) surface.

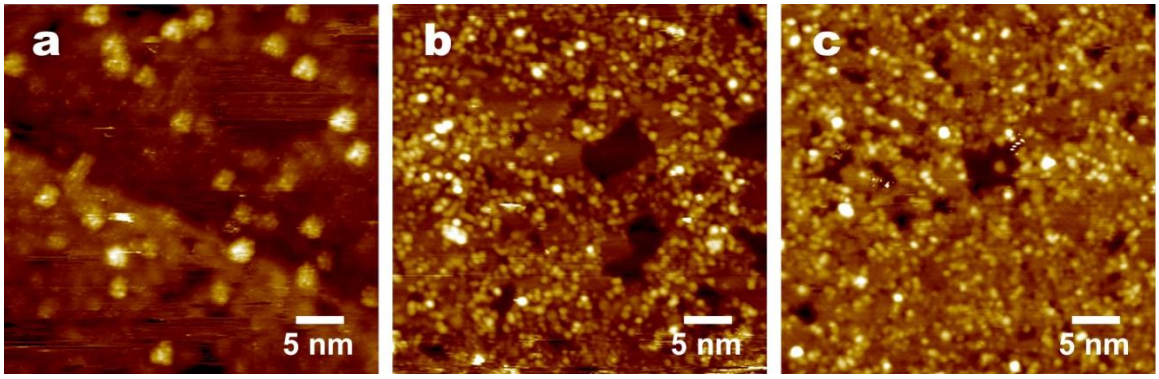

Figure S3. STM images of DNA samples on Au (111) surface. a, DNA prisms are scattered randomly on the gold terrace. b, Controlled sample prepared without annealing. c, Controlled sample prepared with annealing but quickly cool down.

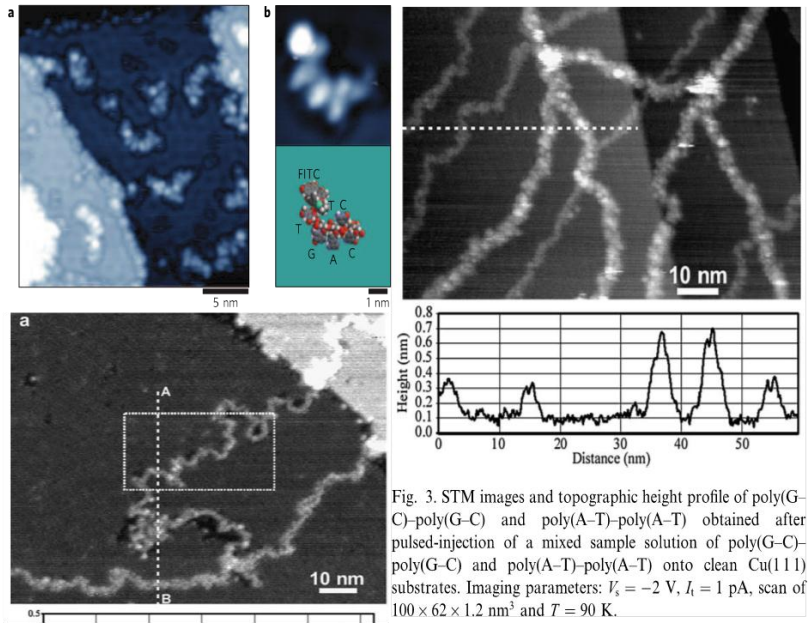

Fig. 3. STM images and topographic height profile of poly(G–C)–poly(G–C) and poly(A–T)–poly(A–T) obtained after pulsed-injection of a mixed sample solution of poly(G–C)–poly(G–C) and poly(A–T)–poly(A–T) onto clean Cu(1 1 1) substrates. Imaging parameters: $V_s = -2$ V, $I_t = 1$ pA, scan of $100 \times 62 \times 1.2$ nm^3 and $T = 90$ K.

Figure S4. STM images of DNA in the literature show that the width of the dsDNA is about 2 nm, whereas the height of dsDNA is only 0.3 nm due to the electroconnectivity problem [S3, S6] With permission of the corresponding author for citing this figure.

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