Preparation of Branched DNA Structures with Long Arms

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Supplementary Information

DNA sequence

The oligonucleotides were purchased from Integrated DNA Technologies, Inc. without further purification. Primer 1: 5'-GAG ACG GTC ACA GCT TGT CT-3'; Primer 2: 5'-Biotin-AGC CAT ACC AAA CGA CGA GC-3'. pUC19 plasmid was purchased from New England Biolabs Inc.

Buffer solution

TBE buffer: tris(hydroxymethyl) aminomethane (Tris, 89 mM), ethylenediaminetetra-acetate (EDTA, 2mM), and boric acid (89mM), pH=8.0 at 25ºC.

PCR Buffer: 50 mM of Tris-HCl (pH=8.0 at 25 °C), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol and 1% Triton®X-100.

Preparation of biotinylated dsDNA by PCR

The PCR reaction mixture contained 2 μM of primer 1 and primer 2, 0.4 ng/μL of template pUC19 plasmind, 2.5 mM $MgCl_2$, 200 μM of each dNTP, 5 units of Taq DNA polymerase (Promega Corporation, Madison, WI, USA) in a total volume of 100 μL Storage Buffer A. The temperature profile consisted of heating to 95 °C for 5 min, followed by cooling to 70 °C for 5 min during which time Taq DNA polymerase was added to the tube (hot start), followed by 25 cycles of 95 °C for 30 s, 55 °C for 1.5 min and 72 °C for 2 min, followed by a final elongation step of 72 °C for 10 min. The PCR product was purified by 1% agarose gel containing 0.5 μg/mL of ethidium bromide. For gel electrophoresis, the running buffer was 0.25 time concentrated as the TBE buffer and was designated as 0.25 X TBE. The major band was sliced and sealed into a dialysis membrane tube filled with 0.25 X TBE buffer. Then then DNA product was electroeluted from the gel blocks and collected. The purified product was quantified by measuring its UV absorbance at 260 nm. Typically, about 5 μg of the product was obtained.

Formation of branched DNA-streptavidin complexes

The biotinylated dsDNA (20 μg/mL) was mixed with streptavidin at molar ratio of 5:1 in 0.25 X TBE buffer. Adjust the Mg^{2+} concentration to 2 mM by adding 1 M magnesium acetate solution. The mixture was incubated at 22 °C for 15 hours and separated by agarose gel (0.5%) electrophoresis on with 0.25 X TBE as running buffer. The desired junctions with different ratio between DNA and streptavidin were sliced separately and electroeluted into 0.25 X TBE buffer at 4 °C.

AFM imaging

A drop of 3 μL DNA junction solution was spotted onto freshly cleaved mica surface, and sat there for 10 seconds. The sample drop was then washed off by 50 μL 1 mM

$Mg(Ac)_2$ solution, and dried by compressed air. DNA samples were imaged by tapping-mode AFM on Nanoscope IIIa (Digital Instruments) with NSC15 tips (silicon cantilever, MikroMasch). The tip-surface interaction was minimized by optimizing the scan set-point.

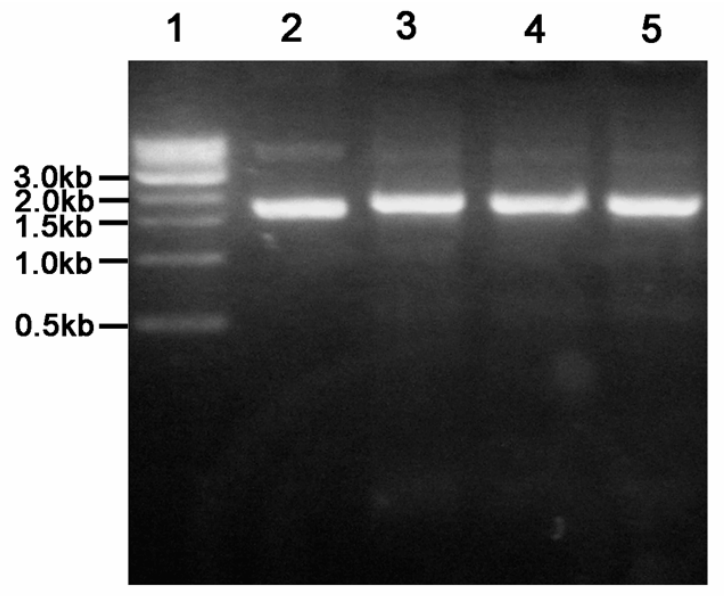

Figure 1S. Agarose gel electrophoresis analysis of PCR product. lane 1: DNA size markers (1 kb ladder), lanes 2-5: PCR products.

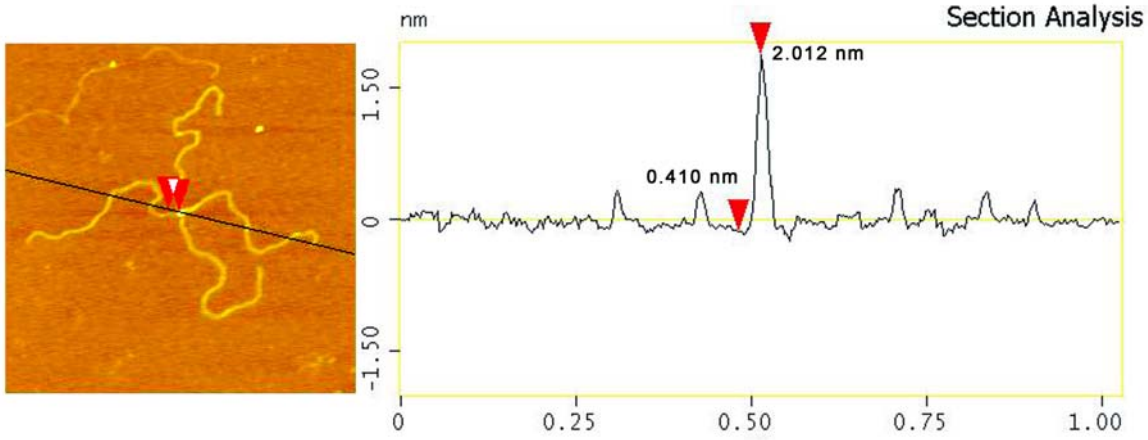

Figure 2S. Height analysis of a four-branched DNA-streptavidin complex. The result shows that the height of the steptavidin core is about 2 nm and the height of the dsDNA branch is about 0.4 nm.

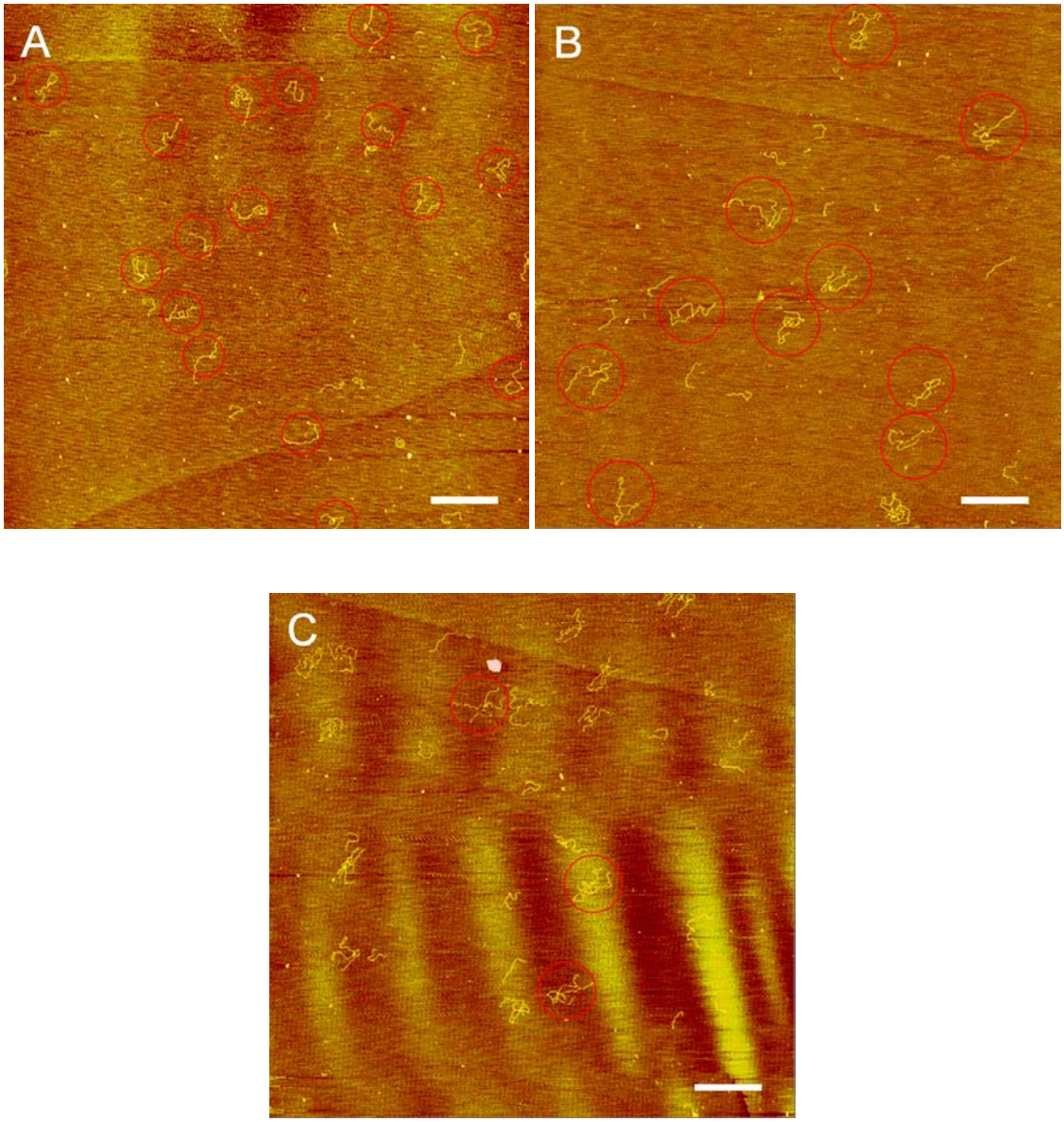

Figure 3S. AFM images of branched DNA–streptavidin complexes with large scanning areas. 2-branched (A), 3-branched (B), and 4-branched (C). Intact branched DNA structures are indicated with red circles. Scale bar is 1 μm.