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Supporting Information

Atomic Force Microscopy-based Single-Molecule Force Spectroscopy Detects DNA Base Mismatches

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Experimental Section

Chemical and Materials

Over 200 short ssDNA include staple strands with 5`terminal extension, and thiolated DNA oligonucleotide (5`-GGTAGTGTAGGACTCCTACGCGAGCGGAGA (T)30-(CH2)6-SH-3`), (5`-CCATCACATCCTGAGGATGCGCTCGCCTC (T)30-(CH2)6-SH-3') were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Original p7249 scaffold of the M13mp18 phage was purchased from New England Biolabs, Inc. (catalog number: # 4040S). All other chemicals used in the experiments were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and used as supplied unless otherwise stated. All chemicals used in this investigation were of analytical grade. All solutions were prepared with Millipore water with a resistivity of 18.2 MΩ.

Composition of Buffer Solution

1×TAE/Mg2+ buffer containing 40 mM Tris, 20 mM acetic acid, 2 mM EDTA, 12.5 mM magnesium chloride at pH 8.0.

Synthesis and Purification of DNA Origami

Using DNA origami as a template, a triangle-shaped DNA origami was constructed described in ref. 1. Briefly, we used a long ssDNA (scaffold), for which the original p7249 scaffold of the M13mp18 phage with a length of ~2.4 μm was folded and stapled into prescribed objects by hundreds of short synthetic DNA oligonucleotides. These synthetic oligonucleotides were typically 20–60 bp long and designed to be complementary to different parts of the scaffold DNA. Several oligonucleotide staple strands were replaced by oligonucleotide staple strands with modified extensions at corresponding sites. Six staple strands (Table S1) were modified with a dumbbell hairpin to provide a reference marker for local height difference. The distance between the strands was chosen to minimize contact between the strands but also maximize the density of the strands in a local region. Taking the strand as a randomly folded chain, we estimated the size of the strand as ~6 nm^2. To purify the DNA Origamis from unbound DNA-oligos, we used Amicon® PCR Centrifugal Filter Devices (Millipore, USA).

Tip Functionalization

AFM tips were stored in a cleanroom environment, washed with ethanol, and dried under a stream of dry nitrogen immediately before use. Next, 100 μL 1× TAE/Mg^{2+} buffer solution (pH 8.0) containing 5 μM thiolated DNA oligonucleotide was added to one clean glass disk before carefully submerging a gold-coated tip. After incubation for 2 h, the tip was rinsed with ultrapure water[1].

FD Measurement

All AFM measurements were performed with a Multimode 8 SPM equipped with a Nanoscope V Controller (Bruker, USA) under 1×TAE buffer. Nanoscope version 8.15 software (Bruker, USA) was used for data collection. PF-QNM mapping was conducted at room temperature using a cantilever (TR400PB, a V-shaped gold-coated silicon nitride cantilever, Olympus, Tokyo, Japan) was modified with thiolated DNA oligonucleotide above-mentioned procedure. The spring constant of cantilever was obtained by built-in cantilever calibration (ramp and thermal tune procedure) in the Nanoscope Software, and resulting spring constants for the cantilevers used were 0.06–0.09 Nm-1. Samples were prepared by first depositing a small drop ~2μL onto the freshly cleaved mica surface and was incubated for 2–5 min. Prior to force measurements, topographic images of DNA origami with extension strands were captured in PeakForce Tapping mode with a scan speed of 1 Hz at a resolution of 256×256 pixels per image to locate the DNA origamis and ensure their appropriate morphology. Once a DNA origami had been located via topographical scanning, the oscillation of the cantilever was stopped, and extending and retracting deflection-separation curves measured between the surface of DNA origami and the gold-coated cantilever were captured under solution using the AFM software. Force measurements were made on DNA origami-free of the mica before making the measurements on the DNA origami. For each DNA origami with an obvious index investigated, multiple contact points covering the extending strands regions were selected for force measurements using the Point and Shoot feature in the AFM software. All force measurements were carried out under HBSS with a trigger threshold of 800 pN. With such a threshold, the cantilever was always on the DNA origami surface. Each force curve was performed at a ramp rate of 0.1 Hz, Ramp size of 150 nm, and a resolution of 4096 points.

Data Analysis

We only considered force traces with rupture events occurring at extensions > 17 nm as reflecting genuine rupture events of the hybridized molecules owing to expectations from contact mechanics. In particular, assuming a Hertz contact model between the tip and sample and based on our previous measurements of the DNA origami elasticity[3], we calculate that during the initial maximal contact between the tip and sample, there is a contact area of radius ~ 13 nm. Thus any molecules on the tip within this radius can interact with the target molecules on the DNA origami (also within this radius). As a result, those hybridizations occurring between molecules not located to the ultimate tip apex are expected to begin to be extended before the tip apex has actually lost contact with the surface. Thus, roughly, the true “zero” point for the extending molecules in this experiment is expected to range over a distance of ~13 nm. Likewise, the distance at which rupture occurs is also expected to range over a distance of ~13 nm (for the same pair of interactors). For a typical ssDNA/dsDNA segment studied here, a force of ~30 pN occurs at an extension of ~30 nm. Thus, with a typical rupture force here of ~30 pN, we considered for analysis only those extensions that are larger than 17 nm (that is, 30 nm minus 13 nm). The initial slowly rising portion of the FDs was compared with what is expected from the extension of a suitable length of ssDNA and dsDNA[4] either in series (for the linker and dsDNA segments) or in parallel (for the mismatched region) as depicted in Figure 3A. For the analysis presented in Figure 3C, we chose only those molecules whose rupture force extensions were within 3 nm of each other in the averages.

Supporting Table

Number of consecutive mismatched bases	Staple strand	Sequence (5`→3`)
0	A15	TCTCCGCTCGCGTAGGAGTCCTACACTACCTTGGAGGGAATTTAGCGTCAGACTGTCCGCCTCC
	A20	TCTCCGCTCGCGTAGGAGTCCTACACTACCTTTTGACGGAAATACATACATAAAGGGCGCTAATATCAGAGA
	A22	TCTCCGCTCGCGTAGGAGTCCTACACTACCTTATTAAAGGCCGTAATCAGTAGCGAGCCACCCT
	A26	TCTCCGCTCGCGTAGGAGTCCTACACTACCTTCACCGTCACCTTATTACGCAGTATTGAGTTAAGCCCAATA
	A27	TCTCCGCTCGCGTAGGAGTCCTACACTACCTTAGCCATTTAAACGTCACCAATGAACACCAGAACCA
	A30	TCTCCGCTCGCGTAGGAGTCCTACACTACCTTGAGCCAGCGAATACCCAAAAGAACATGAAATAGCAATAGC
5	A49	TCTCCGCTCGCGATCCTGTCCTACACTACCTTAGCATGTATTTCATCGTAGGAATCAAACGATTTTTTGTTT
	A52	TCTCCGCTCGCGATCCTGTCCTACACTACCTTCCCATCCTCGCCAACATGTAATTTAATAAGGC
	A56	TCTCCGCTCGCGATCCTGTCCTACACTACCTTACAAGAAAGCAAGCAAATCAGATAACAGCCATATTATTTA
	A58	TCTCCGCTCGCGATCCTGTCCTACACTACCTTAATAGATAGAGCCAGTAATAAGAGATTTAATG
	A61	TCTCCGCTCGCGATCCTGTCCTACACTACCTTGCGCCTGTTATTCTAAGAACGCGATTCCAGAGCCTAATTT
	A62	TCTCCGCTCGCGATCCTGTCCTACACTACCTTTCAGCTAAAAAAGGTAAAGTAATT
6	C15	TCTCCGCTCGCGATCCTCTCCTACACTACCTTTGACCTGACAAATGAAAAATCTAAAATATCTT
	C20	TCTCCGCTCGCGATCCTCTCCTACACTACCTTGAATACGTAACAGGAAAAACGCTCCTAAACAGGAGGCCGA
	C22	TCTCCGCTCGCGATCCTCTCCTACACTACCTTCAATATTTGCCTGCAACAGTGCCATAGAGCCG
	C26	TCTCCGCTCGCGATCCTCTCCTACACTACCTTCTATTAGTATATCCAGAACAATATCAGGAACGGTACGCCA
	C27	TCTCCGCTCGCGATCCTCTCCTACACTACCTTCGCGAACTAAAACAGAGGTGAGGCTTAGAAGTATT
	C30	TCTCCGCTCGCGATCCTCTCCTACACTACCTTTAAAACATTAGAAGAACTCAAACTTTTTATAATCAGTGAG
7	C49	TCTCCGCTCGCGATCCTCACCTACACTACCTTGTTTGCGTCACGCTGGTTTGCCCCAAGGGAGCCCCCGATT
	C52	TCTCCGCTCGCGATCCTCACCTACACTACCTTCGCGCGGGCCTGTGTGAAATTGTTGGCGATTA
	C56	TCTCCGCTCGCGATCCTCACCTACACTACCTTTTAATGAAGTTTGATGGTGGTTCCGAGGTGCCGTAAAGCA
	C58	TCTCCGCTCGCGATCCTCACCTACACTACCTTTGTCGTGCACACAACATACGAGCCACGCCAGC
	C61	TCTCCGCTCGCGATCCTCACCTACACTACCTTTTCCAGTCCTTATAAATCAAAAGAGAACCATCACCCAAAT
	C62	TCTCCGCTCGCGATCCTCACCTACACTACCTTGCGCTCACAAGCCTGGGGTGCCTA
8	B15	TCTCCGCTCGCCATCCTCACCTACACTACCTTGATTAGAGATTAGATACATTTCGCAAATCATA
	B20	TCTCCGCTCGCCATCCTCACCTACACTACCTTTAATTGCTTTACCCTGACTATTATGAGGCATAGTAAGAGC
	B22	TCTCCGCTCGCCATCCTCACCTACACTACCTTTAAGAGGTCAATTCTGCGAACGAGATTAAGCA
	B26	TCTCCGCTCGCCATCCTCACCTACACTACCTTCGGATGGCACGAGAATGACCATAATCGTTTACCAGACGAC
	B27	TCTCCGCTCGCCATCCTCACCTACACTACCTTTAATTGCTTGGAAGTTTCATTCCAAATCGGTTGTA
	B30	TCTCCGCTCGCCATCCTCACCTACACTACCTTTGCTGTAGATCCCCCTCAAATGCTGCGAGAGGCTTTTGCA
9	B49	TCTCCGCTCGCCATCCTCAGCTACACTACCTTTATCATCGTTGAAAGAGGACAGATGGAAGAAAAATCTACG
	B52	TCTCCGCTCGCCATCCTCAGCTACACTACCTTGTACAACGAGCAACGGCTACAGAGGATACCGA
	B56	TCTCCGCTCGCCATCCTCAGCTACACTACCTTCCAAGCGCAGGCGCATAGGCTGGCAGAACTGGCTCATTAT
	B58	TCTCCGCTCGCCATCCTCAGCTACACTACCTTACCCCCAGACTTTTTCATGAGGAACTTGCTTT
	B61	TCTCCGCTCGCCATCCTCAGCTACACTACCTTAAAACACTTAATCTTGACAAGAACTTAATCATTGTGAATT
	B62	TCTCCGCTCGCCATCCTCAGCTACACTACCTTGGCAAAAGTAAAATACGTAATGCC
Marker	M02	AGCGTCATGTCTCTGATCCTCTTTTGAGGAACAAGTTTTCTTGTATTTACCGACTACCTT
	M03	TTCATAATCCCCTTATTCCTCTTTTGAGGAACAAGTTTTCTTGTTAGCGTTTTTCTTACC
	M05	TTTGATGATTAAGAGGCTGATCCTCTTTTGAGGAACAAGTTTTCTTGTGACTTGCTCAGTACCAGGCG
	M06	CCGGAACCCAGAATGGTCCTCTTTTGAGGAACAAGTTTTCTTGTAAAGCGCAACATGGCT
	M10	TGTACTGGAAATCCTCTCCTCTTTTGAGGAACAAGTTTTCTTGTATTAAAGCAGAGCCAC

M11		CACCGGAAAGCGCGTTTCCTCTTTTGAGGAACAAGTTTTCTTGTTTCATCGGAAGGGCGA
Tip-Ex	3'SH	GGTAGTGTAGGACTCCTACGCGAGCGGAGATTTTTTTTTTTTTTTTTTTTTTTTTTTTT-$(CH_2)_6$-SH
Tip-Con	3'SH	CCATCACATCCTGAGGATGCGCTCGCCTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-$(CH_2)_6$-SH

Supporting Figures

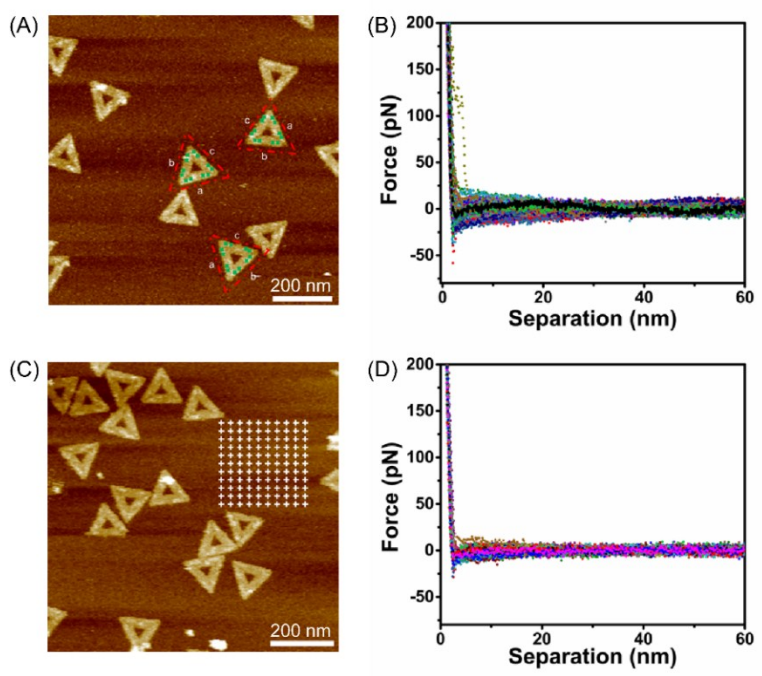

Figure S1. Force curve-based AFM imaging DNA origami using the Au-coated tip modified with the same DNA sequence as the Mis0 on DNA origami. (A) A tapping mode image of DNA origami anchored with different specific DNA strands taken in water. The green cross marks on the DNA origami were assigned using the point and shoot feature of the AFM software. Each green cross mark indicates a location of a force measurement. (B) FD curves showing unspecific adhesion events on the specific sites of DNA origami (All curves were taken at the position of the green cross mark in (A)). (C) Imaging the DNA origami and collecting the force curve on mica using the point and shoot feature of the AFM software. Each white cross mark indicates a location of a force measurement. Repeatedly collect 10 force curves at each cross mark. (D) FD curves showing unspecific adhesion events on mica. (All curves were taken at the position of the white cross mark in (C)). Scale bars correspond to 200 nm.

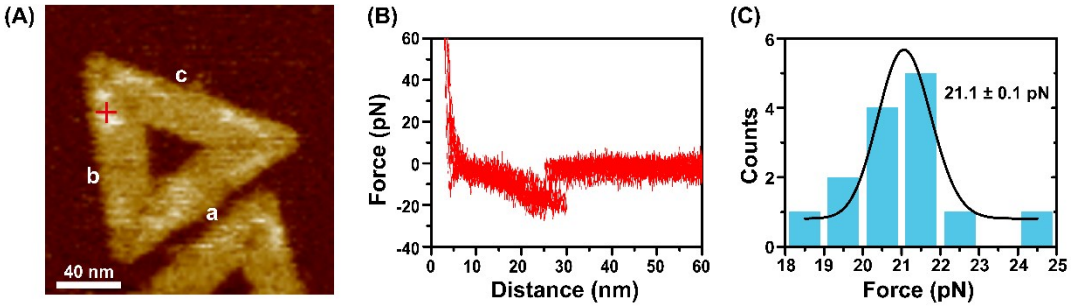

Figure S2. SMFS events at the same location of DNA origami. To further confirm the stability of the ssDNA attached to DNA origami, we performed the consecutive stretching event at the same location of DNA origami for 100 times. After a hundred stretching events, 14 specific rupture force curves were obtained, which meaning that ssDNA can be firmly immobilized on DNA origami.

References

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