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Supporting Information for "Clear-cut Observation of PNA Invasion using Nanomechanical DNA Origami Devices" by Takahiro Yamazaki, Yuichiro Aiba, Kohei Yasuda, Yusuke Sakai, Yusei Yamanaka Akinori Kuzuya*, Yuichi Ohya and Makoto Komiyama*

Detailed Experimental Procedures

Materials. Staple DNA strands were purchased from Sigma Genosys (Japan) or Hokkaido System Science Co., Ltd. (Japan) then used without further purification.

Synthesis of bis-PNA. The bis-PNA was synthesized by Boc chemistry, purified by reversed-phase HPLC, and characterized by MALDI-TOF mass spectrometry (Bruker, AutoFLEX). The detailed protocols were described elsewhere[1].

Preparation of nanomechanical DNA origami devices. Formation of nanomechanical DNA origami was performed with M13mp18 ssDNA (4 nM, Takara, Japan), staples and zipper elements (16 nM for each strand) in a solution containing Tris (40 mM), acetic acid (20 mM), EDTA (10 mM), and magnesium acetate (12.5 mM, 1 X TAE/Mg buffer, 100 μL). This mixture was cooled from 90˚C to 25˚C at a rate of -1.0˚C/min using a PCR thermal cycler to anneal the strands.

Induction of shape transition by PNA invasion. Annealed mixture of nanomechanical DNA origami devices (100μL) was ultrafiltrated by using an ultrafiltration microtube (Amicon Ultra 0.5 mL-100K, Millipore, Ireland) to remove excess staples and zipper elements, and to exchange the buffer of the sample to 1 X HEPES/Mg buffer containing HEPES (5 mM) and magnesium acetate (12.5 mM). After 0 or 1000 eq. of bis-PNA to the four zipper elements on nanomechanical DNA origami devices (final concentration, 4 nM) was added to the solution, the mixture was incubated at 16˚C for 1day.

AFM imaging. AFM imaging was performed on a SPA-300HV system (SII). A mixture containing DNA origami devices and bis-PNA (1μL) was mixed with 1 X TAE/Mg buffer (5μL) and left for several minutes, then deposited on freshly cleaved mica and additional 1 X TAE/Mg buffer (100 μL) was added. Imaging was performed in the fluid DFM scanning mode with a BL-AC40TS tip (Olympus). DNA origami devices in an image were counted as the cross form, when both of the ends were clearly separated AND the levers around the fulcrum were clearly not laid in parallel. They were counted as the parallel form when at least one of the ends was clearly identified to be in head-to-head (the end of a lever close to the concavity) or tail-to-tail (the opposite end of the lever) contact, or into antiparallel form when head-to-tail contact was clearly observed for at least one of the ends. Devices not in any of the above conditions were counted as unclear motifs.

Agarose gel electrophoresis. Agarose gel electrophoresis was carried out on an ice bath using 1.5% agarose gel containing 1 X TAE/Mg buffer under following conditions; 50V, 2.5h. The gel was stained with GelStar (FMC Bioproducts, ME, USA) and imaged on a Typhoon FLA-7000 (GE Healthcare, UK).

Table S1. Sequence and molecular mass of bis-PNA measured by MALDI-TOF Mass Spectrometry.

	Sequence[a)]	Calcd.	Found
bis-PNA	H2N-P (Lys) CCTTTCTCTC-X_3-CTCTCTTTCC (Lys) -H	6050.8	6041.4

a) P and X indicate phosphoserine and 8-amino-3,6-dioxaoctanoic acid, respectively.

1. M. Komiyama, Y. Aiba, T. Ishizuka and J. Sumaoka, *Nat. Protoc.*, **2008**, *3*, 646–654.

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Table S2. Counted numbers of the motifs in AFM images.

	Full Match			Mismatch		
	Before Incubation	Without bis-PNA	With bis-PNA	Before Incubation	Without bis-PNA	With bis-PNA
Cross	17	19	78	11	10	16
Antiparallel	1	3	15	0	1	2
Parallel	73	78	17	76	81	73
Uncear	14	17	37	36	17	16
Sum	105	117	147	123	109	107

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Table S3. Staple strands used to form DNA origami pliers. Other staple strands are listed in the Supporting Information of ref. 8 in the main text.

65long	TCTTAAACAGCTTGATGTGCCGTCGAGAGGGTGAGCCGCC	
66s-PNA-S	GTATAGCCGCTTTCGAGGTGAATTTTTTCTCTCTTTCC	Full match closer
66s-PNA-SM1	GTATAGCCGCTTTCGAGGTGAATTTTTTCTCTGTTTCC	One base mismatch
69long	ACAATGACAACAACCATAGCGGGGTTTTGCTCCAGGTCAG	
70s-PNA-S	GCGGATAAACCGATAGTTGCGCCGTTTTCTCTCTTTCC	Full match closer
70s-PNA-SM1	GCGGATAAACCGATAGTTGCGCCGTTTTCTCTGTTTCC	One base mismatch
73long	GATATATTCGGTCGCTAGGCTGAGACTCCTCACAAATAAA	
74s-PNA-S	ATTAGGATTCGCCCACGCATAACCTTTTCTCTCTTTCC	Full match closer
74s-PNA-SM1	ATTAGGATTCGCCCACGCATAACCTTTTCTCTGTTTCC	One base mismatch
78s-PNA-S	GTATTAAGGAGGCTTGCAGGGAGTTTTTCTCTCTTTCC	Full match closer
78s-PNA-SM1	GTATTAAGGAGGCTTGCAGGGAGTTTTTCTCTGTTTCC	One base mismatch
166s-PNA-S	GTCGAGGTAGAGATAGAACCCTTCTTTTGGAAAGAGAG	Full match closer
166s-PNA-SM1	GTCGAGGTAGAGATAGAACCCTTCTTTTGGAAACAGAG	One base mismatch
169long	GGACATTCTGGCCAACGCCGTAAAGCACTAAAATCCTGTT	
170s-PNA-S	CTAAAGGGCGACCAGTAATAAAAGTTTTGGAAAGAGAG	Full match closer
170s-PNA-SM1	CTAAAGGGCGACCAGTAATAAAAGTTTTGGAAACAGAG	One base mismatch
173long	AGATTCACCAGTCACAAGCCCCCGATTTAGAGCGGTCCAC	
174s-PNA-S	GGAAAGCCGATTATTTACATTGGCTTTTGGAAAGAGAG	Full match closer
174s-PNA-SM1	GGAAAGCCGATTATTTACATTGGCTTTTGGAAACAGAG	One base mismatch
177long	TCAATCGTCTGAAATGGGCGAACGTGGCGAGATCACCGCC	
178s-PNA-S	GAAGAAAGACCTACATTTTGACGCTTTTGGAAAGAGAG	Full match closer
178s-PNA-SM1	GAAGAAAGACCTACATTTTGACGCTTTTGGAAACAGAG	One base mismatch