Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information for

"Allosteric Control of Nanomechanical DNA Origami Devices for Enhanced Target Binding"

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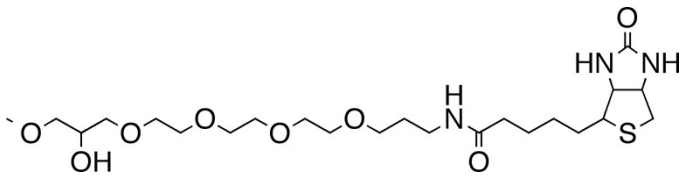
EXPERIMENTAL PROCEDURES

Staple DNA strands and dye-labelled staples were purchased from Sigma Genosys (Japan) and used without further purification. Biotin-TEG and DTPA-HEG modified anchor strands were chemically synthesized using appropriate CPG columns and a phosphoramidite (Glen Research, VA), and purified by reverse-phase HPLC. BSPP-coated 5 nm AuNP was prepared according to the literature (C. Loweth, W. Caldwell, X. Peng, A. P. Alivisatos, P. G. Schultz, *Angew. Chem. Int. Ed.* 1999, **38**, 1808–1812). Formation of nanomechanical DNA origami was performed with M13mp18 ssDNA (4 nM, Takara, Japan), staples and anchor strands (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, 50 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. After excess staples and anchors were removed from the mixture by using an ultrafiltration microtube (Amicon Ultra 0.5 mL-100K, Millipore, Ireland), pre-closing of DNA pliers in the use of G-quadruplex zipper was initiated by adding 1/10 volume of 2 M NaCl to origami solution and kept under room temperature overnight. Capturing of SA in the jaws was then achieved with adding 1 eq. of SA to the solution of DNA pliers, and the mixture was immediately subjected to AFM measurement. For miRNA or AuNP capturing, 2 eq. of the target was used, and the mixture was incubated under room temperature for 2 d before AFM measurement (whereas AuNP capturing is significantly slow probably because of electrostatic repulsion,[14] overnight reaction may be sufficient for miRNA capturing,[5] according to our previous studies). Post-opening of DNA pliers by buffer exchange was achieved by concentrating the mixture with the ultrafiltration microtube and re-suspending it in 1X TAE/Mg buffer three times in ca. 1 h at room temperature (calculated reduction factor of Na^+ was 1/11000). AFM imaging of DNA origami was performed on a SPA-300HV system (SII, Japan) and on a Multimode 8/Nanoscope V system (Bruker AXS). The mixture (3 μL) was deposited on a freshly cleaved mica, additional 1X TAE/Mg buffer (or 1 X TAE/Mg buffer with 200 mM NaCl for pre-closed DNA pliers, 200 μL) was added, and the imaging was done using fluid DFM scanning (or PeakForce Tapping) mode with a BL-AC40TS

tip (Olympus, Japan). Counting of DNA pliers in AFM images were performed as in the previous study.[5]

Biotin-TEG



miR20 and its splitted complements

DTPA-HEG

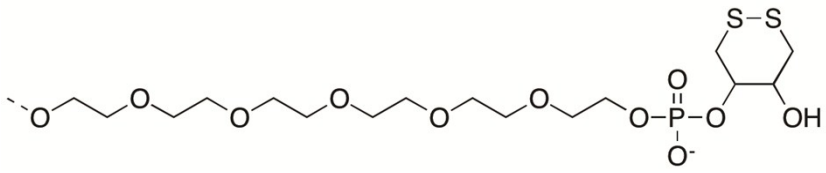


Figure S1. Chemical structure of the ligands.

Table S1. Counted numbers of the motifs in AFM images for Figure 2.

	(i)	(ii)	(iii)	(iv)	(v)
Cross	96	271	26	51	16
Antiparallel	27	79	3	18	3
Parallel	8	25	130	5	50
Unclear	36	47	24	14	12
Sum	167	422	183	88	81

Table S2. Counted numbers of the motifs in AFM images for Figure 3.

	+ Na^+	+ Na^+ + miRNA	+ Na^+ + miRNA - Na^+	+ miRNA
Cross	17	4	22	88
Antiparallel	7	8	5	20
Parallel	78	108	57	55
Unclear	3	14	16	31
Sum	105	134	100	194

Table S3. Counted numbers of the motifs in AFM images for Figure 4

	+ Na^+	+ Na^+ + AuNP	+ Na^+ + AuNP - Na^+	+ AuNP
Cross	17	12	26	11
Antiparallel	7	7	2	3
Parallel	78	104	103	10
Unclear	3	23	33	12
Sum	105	146	164	36

Table S4. Anchor strands used to form DNA pliers. Other staple strands are listed in the Supporting Information of ref. 5a in the main text.

90w bio toe	CGACGGTCAAAACAGATAAAACATCGCCATTAAAAATACCGAAX	X = 3' Biotin-TEG
148w bio toe	CAGCGTGCAAAATACGTTGAGGACTAAAGACTTTTTCATGAGGX	X = 3' Biotin-TEG
91wlongDTPA	AAAACAGATAAAACATCGCCATTAAAAATACCGAAXY	X = spacer C18, Y = 3' DTPA
144wlongDTPA	AAAATACGTTGAGGACTAAAGACTTTTTCATGAGGXY	X = spacer C18, Y = 3' DTPA
90w mir-20	AAAACAGATAAAACATCGCCATTAAAAATACCGAA TTTT CTACCTGCAC	
138w mir-20	TATAAGCACTTTA TTTT ATCCGCGACCTGCTCCGATAAATTGTGTCGAAACACTAAA	