Electronic Supplementary Material (ESI) for Chemical Communications.

DNA Origami Nanocalipers for pH Sensing at the Nanoscale

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1. Materials and methods:

Materials. Reagents for assemble procedures were obtained from commercial suppliers and used without further purification, unless indicated otherwise. Oligonucleotides were purchased from Sangon Biotech (Shanghai, China). Specifically, oligonucleotides longer than 60-nt and those bearing chemical modifications of fluorophores were purified by high-performance liquid chromatography. Carbon nanotubes were purchased from Nanjing XFNANO Materials Tech Co. Ltd.

Design and preparation. The DNA origami was designed using the honeycomb-lattice version of the caDNAno software. The design and sequences were shown in the Table S1. To prevent potential hairpins, homodimers and complementarity to other ssDNA exposed on the structures, unique overhang sequences were added the respective staple strands at the edge of the DNA origami. The hinge consists of two stiff bundles of 23 dsDNA helice. The arms are joined together by flexible ssDNA connections. Three short connections (3nt long) form the hinge axis of rotation, and three longer connections (30nt long) influence flexible motion in one angular degree of freedom. The arms are ~40 nm from the hinge vertex to the opposite end.

Briefly, the mixture of 20 nM single-stranded M13mp18 phage DNA (#P-107, bayou biolabs) and 10-fold excess staple oligos was added into reaction buffer (5 mM Tris, 5 mM NaCl, 1 mM EDTA, and 12.5 mM $MgCl_2$, pH 7.4) in PCR tube. The mixture was then annealed with PCR with the following protocol: incubating the samples at 65 °C for 15 minutes, slowly cooling to 53 °C in a rate of 1 °C /min and incubating at 53 °C for 4 hours, and then cooling to 4 °C.

Agarose gel analysis and image. Typically, the assembled DNA origami nanocalipers were directly loaded on 1% agarose gel with 0.1 % SybrGold and allowed to migrate for about 3 hours at 4 °C (running buffer: 0.5x TBE, 11 mM $MgCl_2$; running voltage: 70 V). The gel was visualized with GE & Amersham Imager 680R in ultraviolet light mode.

Purification of nanocaliper. The self-assembled DNA origami nanocalipers were purified using polyethylene glycol (PEG) precipitation method. Specifically, self-assembly DNA nanocalipers were mixed 1:1 (v/v) with 15% PEG 8000 in purification buffer (5 mM Tris, 1 mM EDTA, and 500 mM NaCl, pH ~7.4) by tube inversion. The solution was spined at 16000 rcf at room temperature for 25 min. After centrifugation, the pellet was resuspended and incubated in target buffer for approximately 20 hours at room temperature.

Assembly of Triplex DNA and Gel electrophoresis. Equivalent Strands Triplex A and Triplex B shown in the Table S1, were dissolved in Tris-HCl buffer (20 mM Tris-HCl buffer, 10 mM $MgCl_2$, pH ~5.0). The mixture was heated to 95 °C in a thermal cycler and then allowed to cool down to room temperature over a period of 2 hours. In the native page, 5μl Triplex DNA mixture (1μM) was loaded in 15% nondenaturing polyacrylamide gel

and electrophoresed for 2 hours at 60V in 1X TAE running buffer (pH ~5.0) with 2.5 mM $MgCl_2$.

Fluorescence analysis. The fluorescence spectrums were acquired with fluorescence spectrometer (FluoroMax-4, Horiba) with a wavelength step of 2 nm/s. For Cy5 in the Triplex DNA and DNA origami nanocaliper, the excitation wavelength was fixed at 570 nm, and the emission spectra was collected in the range of 560-680 nm. The fluorescence intensity at 647 nm was used for further analysis. In the nanocaliper, the Cy3 and Cy5 FRET pairs were mounted 5 base pairs away from the hinge vertex.

CD characterizations. The CD spectrums were acquired with J-1500 Circular Dichroism Spectrometer (JASCO, Japan) in a Quartz cuvette with a 10 mm path length. The final concentrations of the Triplex DNA (pH ~5.0 and pH ~8.0) were about 5 μM in Tris-HCl buffer, respectively. All measurements were carried out at 25 °C.

Particle size determination with Nanoparticle Tracking Analysis (NTA). The high-resolution particle size distribution of nanostructures was measured by Nanosight NS300 (Malvern) at pH ~8.0 and ~5.0, respectively. The final concentration for DNA origami nanocalipers was about 1 nM.

TEM imaging. Before TEM imaging, 10 μL of the DNA samples were deposited on 200 mesh carbon-coated copper TEM grids to adsorb for 5 minutes. And the excess sample solution was blotted away with filter paper. The TEM grids were treated with 2% aqueous uranyl formate solution. And excess stain solution was blotted away with filter paper after 40 seconds and washed with water twice. Grids were imaged with a Thermo Fisher TALOS F200X TEM operating at 120 kV. The angular distribution of the nanocaliper's size were obtained with ImageJ software.

AFM imaging. For imaging of nanocaliper and carbon nanotubes, 10 uL sample solution was deposited on clean mica for 5 minutes. The sample was detected by peak force QNM in fluid mode (Bruke, Multimode8).

DNA sequences. Staple strand sequences for the DNA framework-based nanocaliper.

Hinge 1	0[76]-5[66]	TCGCCATATTTAACTGTAATTGTCCTGATAATATCTCGAGAAGTTT
Hinge 1	0[90]-6[87]	GGCTTAATTCGAGCCTGTTTAGAGCATGTTAAACCATCATTATTTG
Hinge 1	0[111]-13[118]	AAAGCCATATAAAGTTCAGCTTAATCGGCTGTCGGATTATTTCGCAATA
Hinge 1	0[132]-15[146]	TATACAAAAAGTAATTCTGGGCATGATTAGCGAACGTTATTA
Hinge 1	0[153]-6[150]	CCTGTTTTACCGACCTGACCTCAAATCCGTCTGAGGAAAACATAAT
Hinge 1	0[174]-3[174]	AATCATAGGCGTTATATTTTAGAACGCG
Hinge 1	2[79]-16[72]	CAATAGATAATAGGCAGGCAACATCGCAAAGGAAAT
Hinge 1	2[97]-14[94]	AACGCGCCAGTAATGAAA
Hinge 1	2[118]-16[112]	CAACATGTACCGACCGCAGTAAGAAAATGCGACAT

Hinge 1	2[132]-10[136]	ACGACGAAACTATAACCTCCGCTTATCCGGTATAGGAAG
Hinge 1	2[160]-17[150]	TCATCTTCGTGTGAAAGGAGCAAGTTTGCGACAACTCGTATTTAGA
Hinge 1	2[186]-0[175]	TTTTTTCAAATAAATAAGAATTTTTTTAAACACCGG
Hinge 1	3[55]-0[55]	TTTAAAAAACAAGTTTTTTCAACAAACGCTTT
Hinge 1	4[79]-18[72]	ACCGCACTCACCATCCTCCTTTTTAGCAGATAGGAA
Hinge 1	4[97]-17[97]	ACGGGTATAGAAACCAATAGCACCAGAATTCAGCGATTTTGC
Hinge 1	4[118]-7[111]	TTTTTCCTTATCATAAGCAAAACCTCCCGTCTTTC
Hinge 1	4[139]-17[139]	CTTTTTATGTAAATGTTAGAAAAAAGAAGGCAAATAACAACT
Hinge 1	4[160]-17[160]	ATCATAGAATCGCAATACTTCATCAGATTCAATCAGTCAATA
Hinge 1	4[174]-7[174]	GTGAATTATTAAGAGCTTCTGTCAATAT
Hinge 1	4[186]-3[186]	TTTGAGTCAATAAGAAAACTTTTT
Hinge 1	5[67]-22[72]	TTATAGTTGCCCAGCTACAATTTTTATTTATATAAGAAATTTA
Hinge 1	6[86]-21[97]	AAGATCTTACCAACGCTTTACAAAAACGTCAAGGAAGATGATAAT
Hinge 1	6[93]-19[97]	GGTCCGCGCCAGTCAGACCAATAAAAATAGCGTTTACC
Hinge 1	6[149]-21[160]	TAATTAATGGAAACAGTCTGAGCACCAAGTTTGGCAGAAATATTT
Hinge 1	6[163]-19[160]	TCGTCGCTATTAGCGATCAATAACCGTCAGAATCGCCACAGCAAA
Hinge 1	7[55]-4[55]	TTTTGCACTATTTTTTTTTAAGCCCAAGCTTT
Hinge 1	7[81]-20[72]	TGACCTTAAATCAAGATTTTTCATTAATATCCCCACAAAAAGA
Hinge 1	7[112]-11[118]	CATTACATTTAACAAGGCGTTTTAGCGATCAGATATTAACTGGTAAAAC
Hinge 1	7[133]-21[139]	TTGAATTAACAAACATAAAAAGACCAGTGTAAGAA
Hinge 1	7[175]-5[186]	ATGTGAGTGTTTTTTAATAACCTTCGCTGAGAATTT
Hinge 1	8[146]-7[132]	ATGATGAACCTTTTTTTTCCCTTTCTAAGAACGCGATTTCAT
Hinge 1	8[182]-11[182]	TTTAATTAAGGCGTTTTTTCCTTTCAGTATTT
Hinge 1	9[109]-6[94]	AGCGCCTAATTTGCCAGAACGAGCGACTTGCGGGA
Hinge 1	9[130]-22[115]	AACATCAAGAAAACAAAATTAAGAAGCCTTTTCTG
Hinge 1	10[135]-13[146]	CGCTGCGTAGCGAACCACAGTGCCACGCTGAGTCAGTTCTATATT
Hinge 1	12[170]-6[164]	TGTACAGTAATACATCGCGCGCAGTTCATTTCAATTACACATAAATAAA
Hinge 1	12[182]-15[182]	TTTAATCCAATATTTTTTTGGAACTTTGCTTT
Hinge 1	13[88]-7[80]	GTTTATCTTAGTTAAGCGGGTAATTTTGTTTATAAACAGCCATATATCC
Hinge 1	13[147]-4[140]	CCTGATTTGAATAAGGTTTAAGGATTCGCCTGAGGAATCCTTAGACTAC
FRET 1	14[93]-19[87]	ATAGTTTATTGAGGGAAGGTAAATGTATGGGGAGTGAGAACC/Cy3
Hinge 1	15[105]-2[98]	AATCAATTGTTAGCGAGGAAAGCACAATGAAATAGCAATCAAAATGCAG
Hinge 1	15[126]-9[129]	GTTTACCAAGACTCAATACCCCCTACCAAAGAAATATTAGACGAAT
Hinge 1	15[147]-0[133]	ATTTTAAGGAATTATCATCGGTTTGAAAAGTATCATATGCGT
Hinge 1	16[186]-17[174]	TTTAGTATTAGACTTTACACATTTGA
Hinge 1	17[72]-13[87]	AATGAATTTTCTATTGACGACACCACGGAATAACATACATCAAA
Hinge 1	17[98]-15[104]	TAAACAAATTGAGGTTGTCAC
Hinge 1	17[119]-15[125]	TCTTTAGCAAAAGGTCATATG

Hinge 1	17[140]-19[129]	AATAGATAAATCCTTTGCCCGCCAAAGAGAGCACTCAACAGTCCTG
Hinge 1	17[151]-23[153]	GCCATATCTGGAGCCAGTTAAAAACACAGACTTCACCATCTATCA
Hinge 1	17[161]-12[171]	GATAATAAACAATTAGTAACATTATCATAAAGAAAATTCATCTGAT
Hinge 1	18[174]-19[186]	CAAATATTCTAAAGCATCACCTTTTT
Hinge 1	18[186]-17[186]	TTTGCTGAACCTGGATTTAGATTT
Hinge 1	19[72]-0[77]	CAACACTATCATAATAGAAAGCCGAAAAAGGTGAGGCATTTTGAGAA
Hinge 1	19[88]-23[97]	CTCGAGAGGCCCCCGGTTTGTATACAGTTTGGAACAAG
Hinge 1	19[98]-0[91]	AGACGACCAACAGTGGAAACCAAACGTAAAGAGAAACGCTCAACAGTAG
Hinge 1	19[119]-0[112]	AACACCGTGAAAGGATAACGGCTTATTAAAAAGGTATTCTTACCAGTAT
Hinge 1	19[130]-23[139]	CAACCAGCAGTGAAAGCAATAAAAACGTCAAAGGGCGA
Hinge 1	19[161]-0[154]	TGAAAAACAAACCCGATGGCACCACCAGTAAATAAATTACTAGAAAAAG
Hinge 1	20[186]-21[174]	TTTGCGCGAACTGATAGCCGCTATTA
Hinge 1	21[72]-2[80]	CAATCATATGTATTTTGCAGAATTGACCGAAGCAATTTACTCAA
Hinge 1	21[98]-9[108]	CAGAAAAAAAAACCATAAGAGCAAGAAACAACACCCAAAT
Hinge 1	21[119]-2[119]	TCTGACCAAGATAAAGAAATATATCAAAGTTATATCAATAAA
Hinge 1	21[140]-2[133]	TACGTGGTACCGAAATTTTCATGGAAGGGCTGATGAAATTTAATTCCAG
Hinge 1	21[161]-2[161]	TTGAATGCTAAAACTGAATATTTGGATTAGACAAAGTTAATT
Hinge 1	22[174]-23[186]	TGGATTACATTTTGACGCTCAATTTT
Hinge 1	22[186]-21[186]	TTTCGTCTGAAAGTCTTTAATTTT
Hinge 1	23[72]-4[80]	TGAGTGTTGTTCAGCAAATACGATTTTGAGCGCCGTAGGAAAGT
Hinge 1	23[98]-4[98]	AGTCCACCAAAAACAAAATGATGAACAACAATAGCTCCAAGA
Hinge 1	23[119]-4[119]	GACTCCAGGGACATACAGAGAGGGAGAATAGAAGGGCTTAGG
Hinge 1	23[140]-8[147]	AAAACCGGTCACACCATTGCTTTGAATAAAAGAAG
Hinge 1	23[154]-4[161]	CATGGAAATACCTATTTACATACAAAATGGAGAAAAGCTTAGTATCAAA
Hinge 2	28[97]-47[97]	AAATCAAATCAGCTTAAATGTTTGATAAAACTAAAATAGTAG
Hinge 2	28[118]-47[118]	GAAATCGTAGGAACGTAGCCAGGATGGCCTCAACAAATAAAT
Hinge 2	28[139]-31[139]	ATCCTGTTTGTTTTTCTTTTCAAAGGCTATCAGGTAAGAGAA
Hinge 2	28[160]-47[160]	GGTTTGCAGACGGGCGGTTTGCAAGGATTTATGACAATAAAG
Hinge 2	28[193]-29[193]	TTTCTGAGAGAGTTGCAGCCCTTCACCGCCTGGCCTTT
Hinge 2	29[72]-47[76]	CGCATCCGTCGGGAGTACCAGTTTCAAAGGTGG
Hinge 2	30[97]-49[101]	ATGAACGAATACTGTCAAAAAATGAGGAACACTAAAACTTTGAAAG
Hinge 2	30[118]-49[118]	CTGGAGCATATTCAGAAAACGGGGTAAAAAACGAAAACGGTG
Hinge 2	30[163]-49[164]	ATTTTTGAGACAAATCAATGTGTAAACACCAGTAACAAAAGAGTAATCT
Hinge 2	30[193]-31[193]	TTTGATAAATTAATGCCGGTTCAACCGTTCTAGCTTTT

Hinge 2	31[72]-50[80]	TTTAGTGACTATTTGAGGAACCCCCACATAAGGGAACCGACTTG
Hinge 2	31[140]-34[140]	AGGCCGGCAGTTGAGATTTAGAGTAAGAACTGGCTGCCTGTA
Hinge 2	32[86]-28[72]	ACGAGCGTCCGTAATCGTTGTTAAAAGAATAGCCCGAGATAGG
Hinge 2	32[97]-51[104]	CAAAAGGAAAAAAGCGATAGTACCCTCAGTCAGACCCCGTATAAACAGT
Hinge 2	32[118]-51[125]	ACTAATGCTTTAATATTTCTTAGAGCCGATTGACACCCTGCCTATTTCG
Hinge 2	32[149]-28[140]	CATAGACAGTGATCTACACCAGTGCCCAGCAGGCGAAA
Hinge 2	32[193]-33[193]	TTTAAACGAACTAACGGAAAAAATCTACGTTAATATTT
Hinge 2	33[72]-51[87]	TCACGCAACAACGCCGCCATTCACAATCAGTGCCTTGAGTAACA
Hinge 2	33[109]-28[98]	AGCCAGATACGTCATAAAAACAAGTAACCAAGCAAAATCCCTTAT
Hinge 2	33[172]-28[161]	AGACAACATTTATGATAAGAGGGTTGATTGCAAGCGGTCCACGCT
Hinge 2	34[97]-39[87]	GTTTTGTTTAGAGCCCGTAATCAGTAGCAACCGCCTGCGCCGCTTT
Hinge 2	34[118]-39[111]	TAGTTAGGTAGCACATGAAACCATCGATCACCCTCAAACAGCATTAAAC
Hinge 2	34[139]-32[150]	GCATTCCAGCGCCACCCTCAGAGCCACCCTACAACCATTATAGATT
Hinge 2	34[160]-38[150]	GTACAAAACCCTCAACCCTCAGAACCGCGTTGATAGAAT
Hinge 2	34[193]-35[193]	TTTCATGTACCGTAACACTAAGCCCAATAGGAACC???
Hinge 2	35[72]-40[66]	GACTTTCAAGTTTGCCTTTCCTCAGACATCGCCAGAGGCTTATA
FRET2	35[84]-32[87]	TTGGGAACGTCTTTTCTCCAAAATT/Cy5
Hinge 2	35[126]-30[129]	ATTAGCAACAGACAGTTTATCGAATACCCCCCTCACATT
Hinge 2	36[20]-37[20]	AGCCCCCTTATTAGCGTTTTTTTTTGCCATCTTTTCAT
Hinge 2	36[23]-43[24]	CATAATCAAACAGGGAGAGCAGCGGAAGCCCCTTCAAAGGTCACGCTGC
Hinge 2	36[48]-41[45]	GCGCGTTAGCCACCATATTCGAACGAGGTTGCATCAAGC
Hinge 2	36[139]-41[129]	AGAACGCCGGAAACAACCACCTGCTTTCATGCCACCTTTAAATTAA
Hinge 2	36[186]-39[174]	TTTTACTCAGGAGGTTTAGAATAGGTAATTTCATAGTAAA
Hinge 2	38[20]-39[20]	TTAAAGGCCGCTTTTGTTTTTTCGGGATCGTCACCCTC
Hinge 2	38[34]-51[34]	AGGCTTGATCACCGGCAGTCTTTTGATG
Hinge 2	38[149]-43[153]	TACGACGAGAGGTAAAGTTCAACGCGTATTGCCAGTCG
Hinge 2	38[156]-51[160]	TGTTAAGTATTTTTGCTTTAAGAG
Hinge 2	39[60]-51[62]	TACCACGCATAACCGATACCGGAAATTAAAGTGGTAATAAGTTTT
Hinge 2	39[88]-45[94]	TTCTCAGGTCGCTCCTTGAGCGAGCGGCACCGCTTCTGTCTTCGCTCAT
Hinge 2	39[140]-36[140]	TTGCCCTCTTATGCGATTTCTACGCCGTCGAGAGGCACCCTC
Hinge 2	39[175]-37[186]	TTGGGCTTGTTTTTTAGATGGTTTGTATCACCGTTT
Hinge 2	40[20]-41[20]	GAAAGACTTCAAATATTTTTTTCGCGTTTTAATTCGAG
Hinge 2	40[55]-48[52]	AAGCGGAGTAGCAACGCG
Hinge 2	40[65]-45[76]	GTCGATTAGAATTCTCCATCGCACTCCAGCCGCGAAAGGTACCGA
Hinge 2	40[111]-45[118]	AGAATGATTTTTGCGCTTTCAAAACCAGGCAAAGCGAAGGGCGTGTGAA
Hinge 2	40[186]-43[174]	TTTATTTTAAATGCAATGCTTTAGAACGCGCGGCCAGCTG
Hinge 2	41[32]-38[35]	ACCAGTAGATATAACCTGTTTAGCCAACGGAGCATCGGGTCGCTG
Hinge 2	41[46]-45[55]	AAACGGATTGGACAGTATCGGCCTAGGCGATTCGACTC

Hinge 2	41[130]-42[140]	TTGCGTCTGGGCCATTCAGACTGCCCGCTTTGGCGCCA
Hinge 2	41[165]-38[157]	ATTCTGAGTACCATCAAATTACAGGTAGAAACCAGTCATCAT
Hinge 2	42[20]-43[20]	TTGGTGTAGATGGGCGTTTTTTCATCGTAACCGTGCAT
Hinge 2	42[55]-39[59]	CAAACGGCTCCAACTGATTCCTCATTTGGGGCGCGTACCAAGCGGC
Hinge 2	42[139]-39[139]	GGGTGCGCTAGAAGCCTTTATATTCAAAAGGGTTGTAAAGGC
Hinge 2	43[25]-45[34]	CAGTTTGAGGCCAGGGTCCAAGCT
Hinge 2	43[154]-41[164]	GGAAACCTAACTCAGCCGGAAGCATAAAAAAAACAAAAA
Hinge 2	43[175]-41[186]	CATTAATGATTTTTTATCGGCCAACCCTCATATTTT
Hinge 2	44[20]-45[20]	GTCACGACGTTTTTTTTGTAAAACGACG
Hinge 2	44[86]-30[72]	ACGCCAGCTGAGCTTTCTAACAACTAAATTTTAAAACTAGCAT
Hinge 2	44[132]-43[125]	TGCGCAACTGTTGGGCCATTC
Hinge 2	44[182]-47[182]	TTTGGGTGGCCTGTTTTTTATCGGGCTAATTT
Hinge 2	45[35]-48[31]	TGCATGCGCGAACGAGACCGGAAAAAGATTAAGAGAAAGACAGATT
Hinge 2	45[56]-40[56]	TAGAGGATAACAGTAGGTCAGAGAAGCA
Hinge 2	45[77]-32[72]	GCTCGAAGTCTGGATTTAATTTTTACCCACTGGATAGGCATAGTAAG
Hinge 2	45[95]-32[98]	GGTCATAGCTAATATGCGAGGTCACCATAAACGGAATCATAACGC
Hinge 2	45[109]-30[98]	CCTGATCGGTGCGGGCCGTGCCGGTCAACATCATTTTTAGAATCG
Hinge 2	45[119]-41[125]	ATTGTTAGCTGTAGTTAGAGC
Hinge 2	45[140]-47[139]	CACACAATACTTTTGCGGGGAAAAGAAT
Hinge 2	46[20]-47[17]	GACCATTAGATTTTTTTACATTTCG
Hinge 2	46[44]-41[31]	TCTCTGCAGGTAAGTTGGGTAACGGGACGACACCGTAATGGGATAGCGA
Hinge 2	46[65]-42[56]	ATATCCCCGGGGGGATGTGCTGCACAGGAAGGTGGGAA
Hinge 2	46[170]-30[164]	ACCGTGTAAACCTAATGAGTGAGCTGTCGTGGGAGAGGCAACAGCAGCT
Hinge 2	47[77]-34[72]	CATCAATATCTTTGCTAAAGAACAATGATTGAAAACCAGACGTTAGT
Hinge 2	47[98]-34[98]	TAGCATTAAAGAATAGTTTCCTTGATACGCTCCAAATCTAAA
Hinge 2	47[119]-39[125]	CATACAGCAACCTAATACGTA
Hinge 2	47[140]-50[136]	TAGCAAACATTCAGTGAATCGATAGGCTGGCTGACAGGCGGATAAG
Hinge 2	47[151]-45[139]	AGCCCTGTAACATACGACATTAATTGCGTTGCGCTCGCACAATTC
Hinge 2	47[161]-34[161]	CCTCAGAAATCAACGAACGAGACTTTAAGGACGTTGTCACCA
Hinge 2	48[17]-49[20]	GATAAATT??????GTGTCGAAATC
Hinge 2	48[30]-36[24]	TGTTGCTCCATGTTACTGGAAAGCGAACCAGTTCATCGGCATTTTCGGT
Hinge 2	48[51]-36[49]	AAAAACGAGGCGCAGACAATCCTCCCGCCTCAGCGTCAGACTGTA
Hinge 2	48[182]-51[182]	TTTATTCACGGATTTTTTTAGGATAGAGATTT
Hinge 2	49[102]-33[108]	AGGGAGGCAGGAGCCACAGCAGCACAGCAAAATCACCACGTAACGAAGG
Hinge 2	49[119]-35[125]	TACAGACCGCCAGCCCACCAGGTCACCACATTACC
Hinge 2	49[165]-33[171]	TGAAGCGGGGAGCCCGGTACCGCCTTTTCAGGGATAGCGAGTTTCGGGA
Hinge 2	50[20]-51[20]	TACCGTTCCAGTTTTTTTAAGCGTCATA
Hinge 2	50[79]-35[83]	ATACCCTCAGGACAGAAGAGCCAT

Hinge 2	50[135]-47[150]	TCATTATTCTGAAACATGAAAGTACAGTACCCTTCATCAGCTGCTATTA
Hinge 2	51[21]-48[18]	CATGGCTCTGAATTCGCGACCATCATCGCCT
Hinge 2	51[35]-46[45]	ATACAGGAGTGTACCCAGAATTAGCCGGCAAAGTATATATTTCAAT
Hinge 2	51[63]-46[66]	AACGGGGACAAATAGGTCAATGCGATTAAGCTGAATTCC
Hinge 2	51[88]-44[87]	GTGGATTGGCACTGACCAACACTCTCTACTAGTACGGTTTCGTAATATT
Hinge 2	51[105]-45[108]	TAATGCCGGAGGTTACAGATGAGAGGCAAACATCCTGTTTTAGTTT
Hinge 2	51[126]-45[132]	GAACCTAGAGCCGCCAGGCGCAAGGCACGCAAGGCATATAATTCCGCTC
Hinge 2	51[161]-46[171]	GCTGAGACTCCTCATAGGATTCAAGAACTTACCCAGCATAAATTGT
A		AGGAGAAAGGAGAGAG
B		6-FAM/CTCTCTCCTTTCTCCTGTACATCCTCTTTCCTCTC/BHQ1
C		GGTGGATTGACGGATTCTCCGTGGTTGCGAACGA
Complementary strand 30[128]-28[119]		GCCAAAATAATTGATGGTGGTTCCAAACCCCAGGT/BHQ1
Complementary strand 38[125]-34[119]		GAGGTGATGTATCGGCCCTCAAAACCCCAGGT/BHQ1
Complementary strand 40[125]-32[119]		CAGTTCATTGAATCACATTCAAAACCCCAGGT/BHQ1
Complementary strand 42[125]-30[119]		CCTTCCTGCCATCATGAGAGTAAACCCCAGGT/BHQ1
Triplex DNA 16[111]-17[118]		TCAACCGCTTTATCTAAAATATTTTGGGGAGAGGAGGAAGAAAAGAGAGA
Triplex DNA 18[118]-19[118]		AATTGAGGAAGGTTGAGCGGTCAGTATTTTTTGGGGAGAGGAGGAAGAAAAGAGAG A
Triplex DNA 20[118]-21[118]		AACAGAGGTGAGTAGCAGATAGAACCCTTTTTGGGGAGAGGAGGAAGAAAAGAGA GA
Triplex DNA 22[114]-23[118]		GCCAACAGCCTATTAAAGAACGTGTTTTGGGGAGAGGAGGAAGAAAAGAGAGA
Triplex DNA 30[128]-28[119]		GCCAAAATAATTGATGGTGGTTCCTTTTTTCCCCTCTCCTCCTTCTTTTCTCTCTATTA TCTCTCTTTTCTTCCTCCTCTCCCC
Triplex DNA 38[125]-34[119]		GAGGTGATGTATCGGCCCTCATTTTTTCCCCTCTCCTCCTTCTTTTCTCTCTATTATCT CTCTTTTCTTCCTCCTCTCCCC
Triplex DNA 40[125]-32[119]		CAGTTCATTGAATCACATTCATTTTTTCCCCTCTCCTCCTTCTTTTCTCTCTATTATCTC TCTTTTCTTCCTCCTCTCCCC
Triplex DNA 42[125]-30[119]		CCTTCCTGCCATCATGAGAGTTTTTTTCCCCTCTCCTCCTTCTTTTCTCTCTATTATCTC TCTTTTCTTCCTCCTCTCCCC
Free DNA framework 16[111]-17[118]		TCAACCGCTTTATCTAAAATA
Free DNA framework 18[118]-19[118]		AATTGAGGAAGGTTGAGCGGTCAGTATT
Free DNA framework 20[118]-21[118]		AACAGAGGTGAGTAGCAGATAGAACCCT
Free DNA framework 22[114]-23[118]		GCCAACAGCCTATTAAAGAACGTG
Free DNA framework 30[128]-28[119]		GCCAACAGCCTATTAAAGAACGTG
Free DNA framework 38[125]-34[119]		GAGGTGATGTATCGGCCCTCA
Free DNA framework 40[125]-32[119]		CAGTTCATTGAATCACATTCA
Free DNA framework 42[125]-30[119]		CCTTCCTGCCATCATGAGAGT

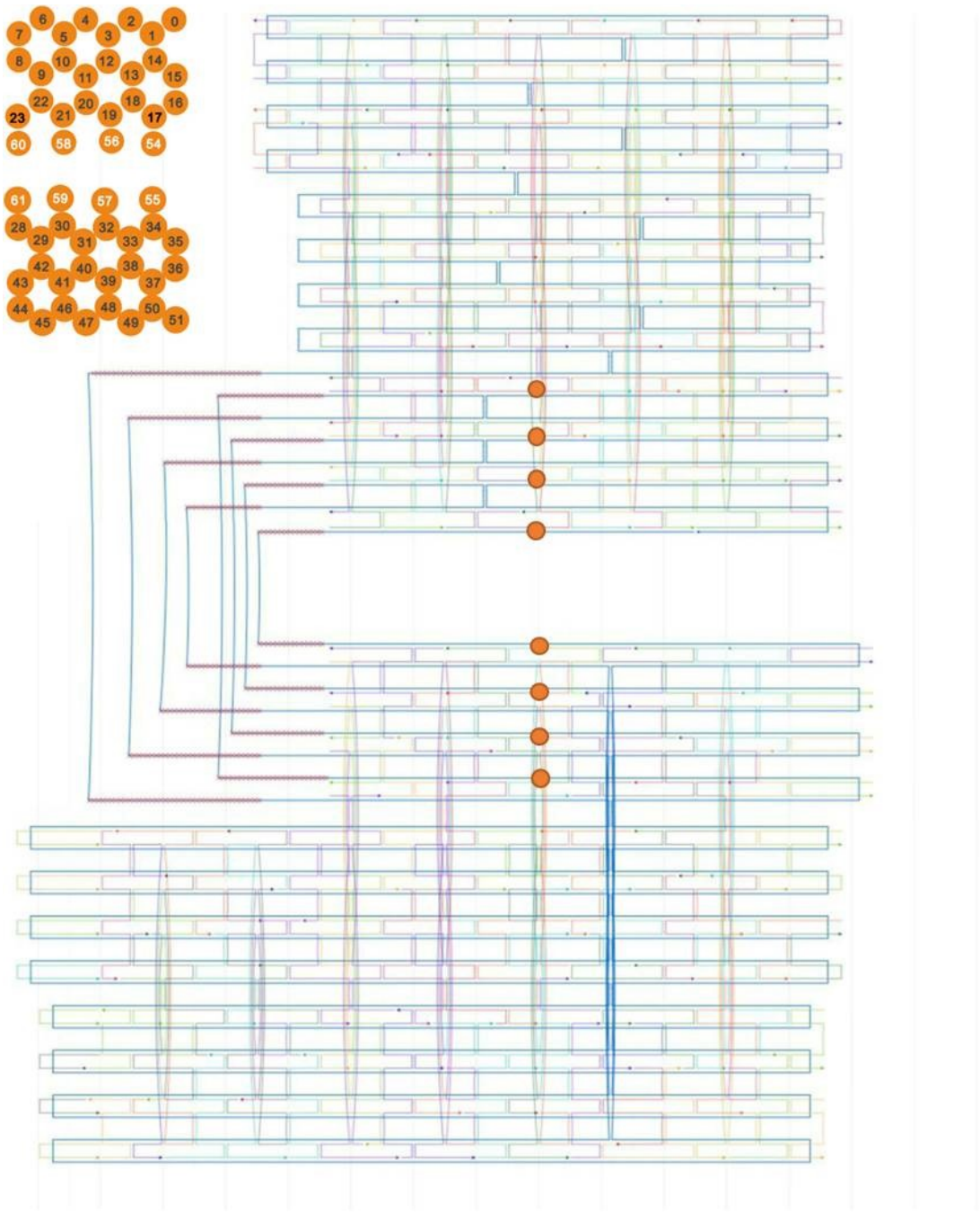

Figure S1. Design diagram of the nanocaliper using caDNAno software. The orange circles indicate the attachment sites for triplex DNA and ATP aptamer.

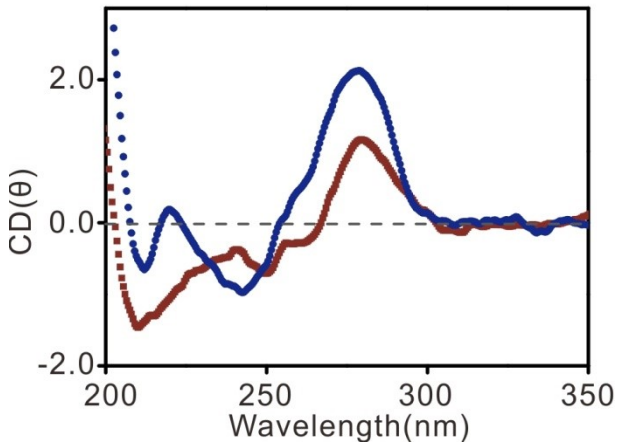

Figure S2. The CD spectral of the triplex DNA at pH 8.0 (blue) and 5.0 (red), respectively.

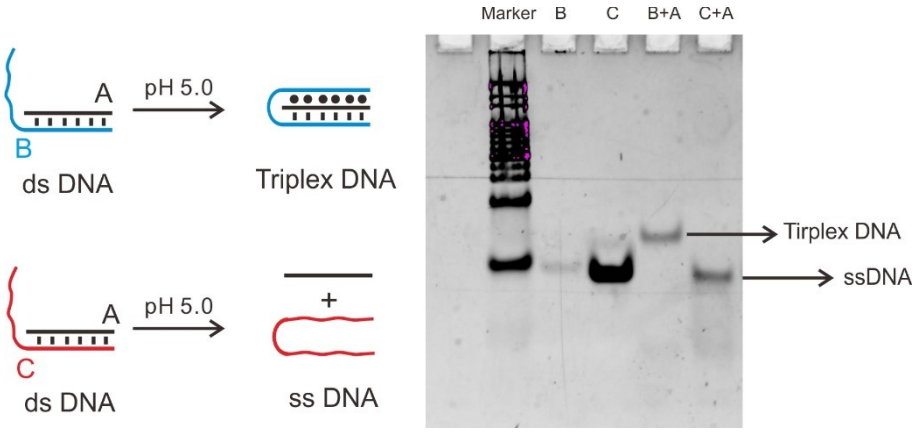

Figure S3. **The electrophoresis analysis of the Triplex DNA.** The ssDNA A&B are synthetic single strand for self-assembly triplex DNA. The DNA C is a random sequence of the same length (DNA B). These DNA mixtures of A+B and A+ C were self-assembled at pH ~5.0.

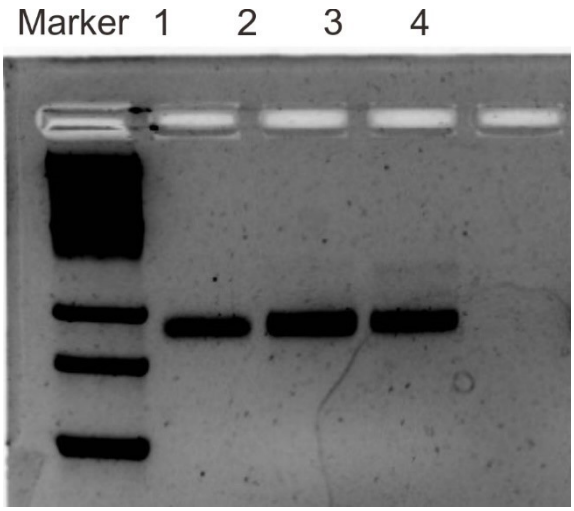

Figure S4. Agarose gel electrophoresis results for nanocalipers in different solution, respectively. Lane 1, Marker; Lane 2, M13; Lane 3, nanocaliper in Tris buffer (5 mM Tris, 5 mM NaCl, 1 mM EDTA, and 12.5 mM $MgCl_2$, pH ~7.4); Lane 4, nanocaliper incubated for 30 mins in weak acidic solution (5 mM Tris, 5 mM NaCl, 1 mM EDTA, 12.5 mM $MgCl_2$, and pH ~5.0).

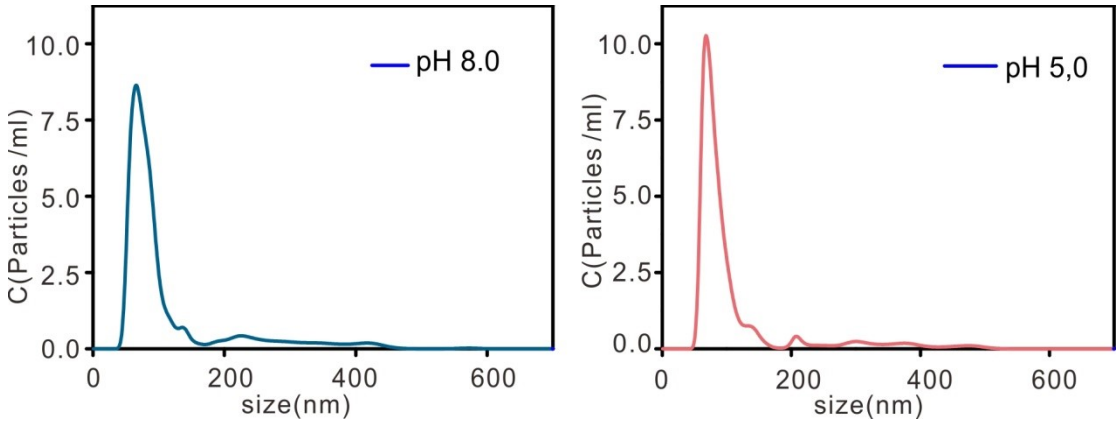

Figure S5. Particle analysis for the nanocalipers at pH 8.0 and 5.0, respectively. These NTA analysis demonstrate that nanocalipers exhibited excellent stability and dispersion.

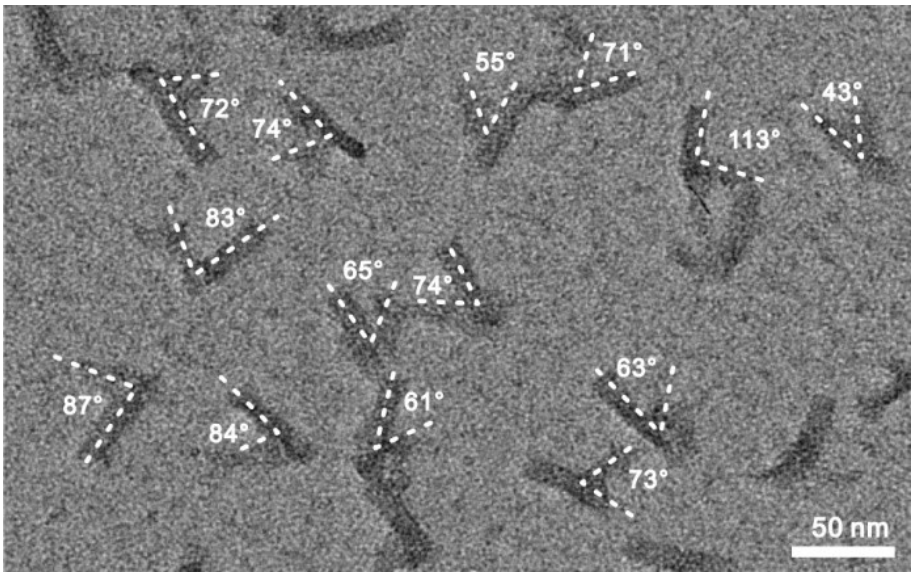

Figure S6. Hinge angle analysis for the nanocaliper in the TEM images in Tris buffer (5 mM Tris, 5 mM NaCl, 1 mM EDTA, 12.5 mM $MgCl_2$, and pH ~7.4).

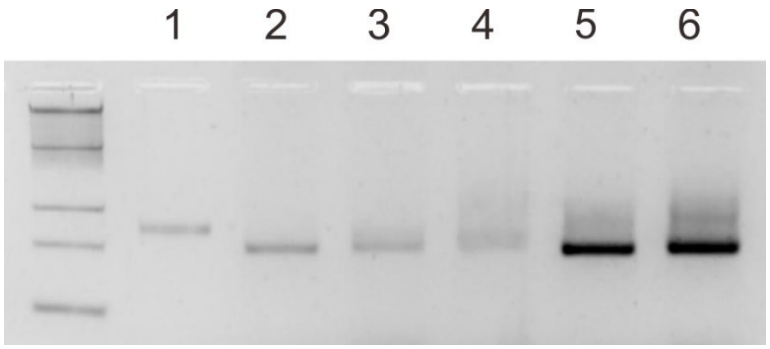

Figure S7. Agarose gel electrophoresis results for DNA origami nanostructures in various conditions. Lane 1, M13. Lane 2, free DNA origami. Lane 3, nanocaliper incubated with additional 40 mM Mg^{2+} (5 mM Tris, 5 mM NaCl, 1 mM EDTA, 40 mM $MgCl_2$, and pH ~7.4). Lane 4, nanocaliper incubated with additional 800 mM Na^{+}(5 mM Tris, 800 mM NaCl, 1 mM EDTA, 12.5 mM $MgCl_2$, and pH ~7.4). Lane 5, nanocaliper incubated with additional 5% PEG (5 mM Tris, 5 mM NaCl, 1 mM EDTA, 12.5 mM $MgCl_2$, 5% PEG, and pH ~7.4). Lane 6, origami incubated with weak acid ((5 mM Tris, 5 mM NaCl, 1 mM EDTA, 40 mM $MgCl_2$, and pH ~5.0).

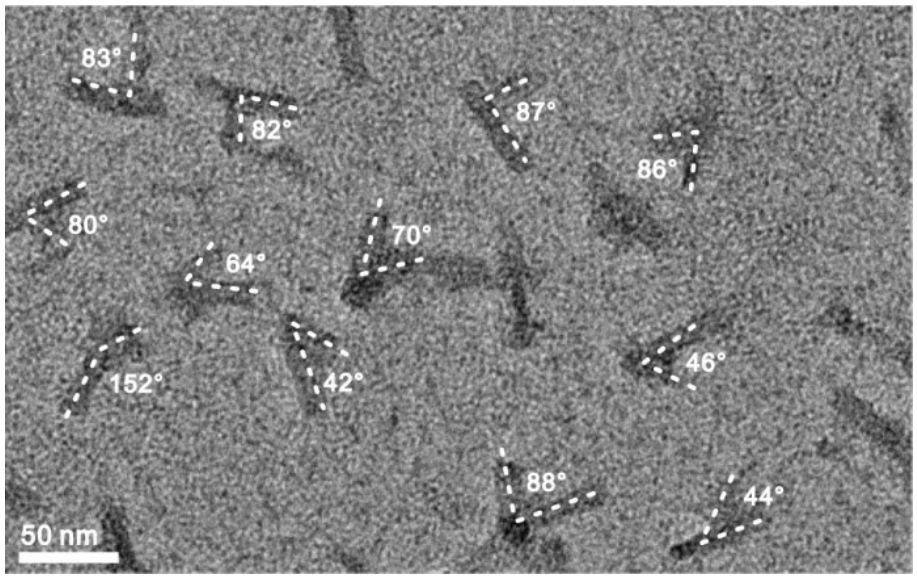

Figure S8. Hinge angle analysis for the DNA origami nanostructures in the TEM images. The mixture of nanocalipers were incubated at high Mg^{2+} concentration condition (5 mM Tris, 5 mM NaCl, 1 mM EDTA, 40 mM $MgCl_2$, and pH ~7.4) for half hour.

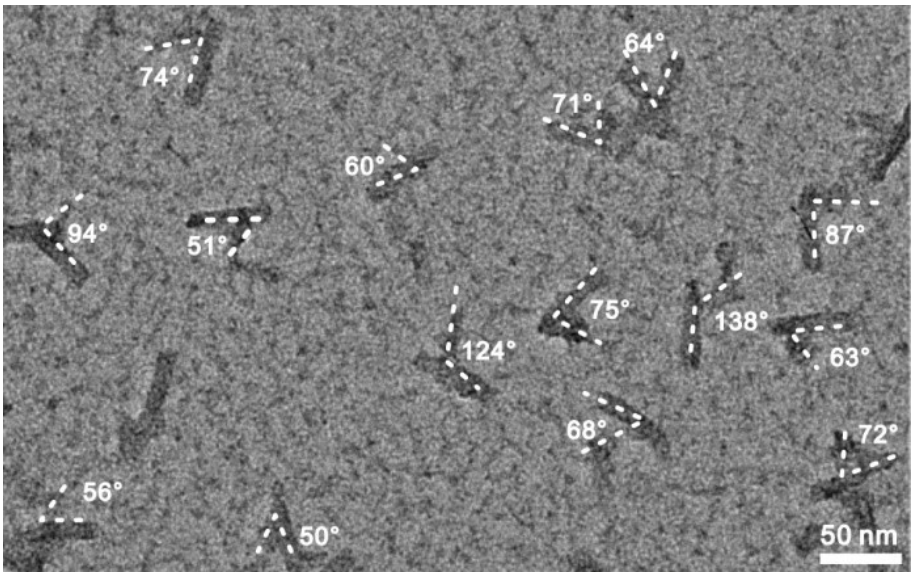

Figure S9. Hinge angle analysis for the DNA origami nanostructures in the TEM images at high Na+ concentration condition. The mixture of nanocalipers were incubated at high Mg^{2+} concentration condition (5 mM Tris, 800 mM NaCl, 1 mM EDTA, 12.5 mM $MgCl_2$, and pH ~7.4) for half hour.

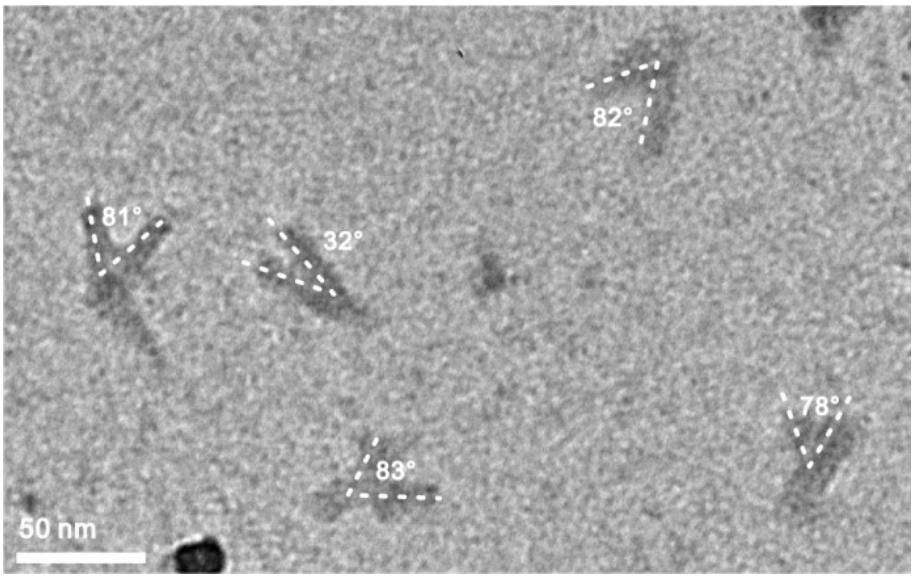

Figure S10. Hinge angle analysis for the DNA origami nanostructures in the TEM images at high molecular crowding condition. The mixture of nanocalipers were incubated in the high crowding buffer (5 mM Tris, 5 mM NaCl, 1 mM EDTA, 12.5 mM $MgCl_2$, 5% PEG, and pH ~7.4) for half hour.

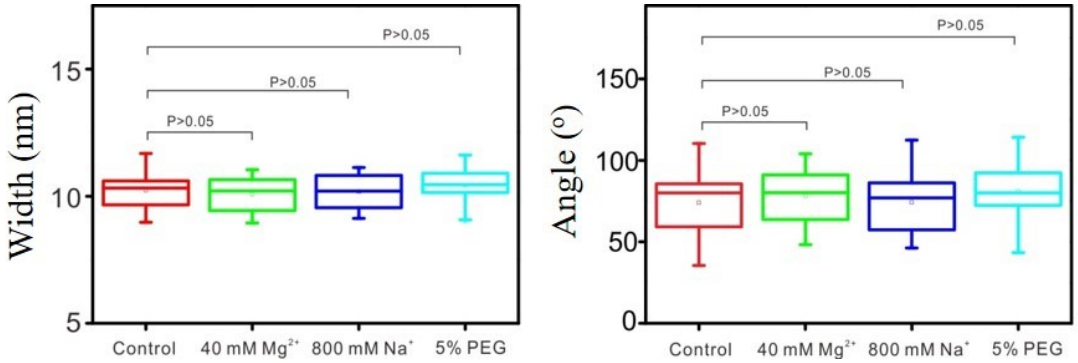

Figure S11. Comparison of the hinge angle and arm width of DNA origami nanostructures with additional 40 mM Mg^{2+}, 800 mM Na^{+}, 5% PEG in Tris buffer (5 mM Tris, 1 mM EDTA, and pH ~7.4), respectively. P value was calculated by One Sample-t Test.

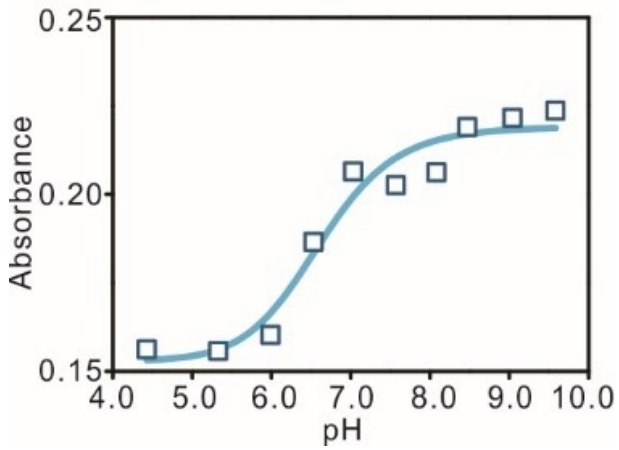

Figure S12. Characterization of the Triplex DNA formation in different pH with absorbance (λ_{abs}=260 nm).

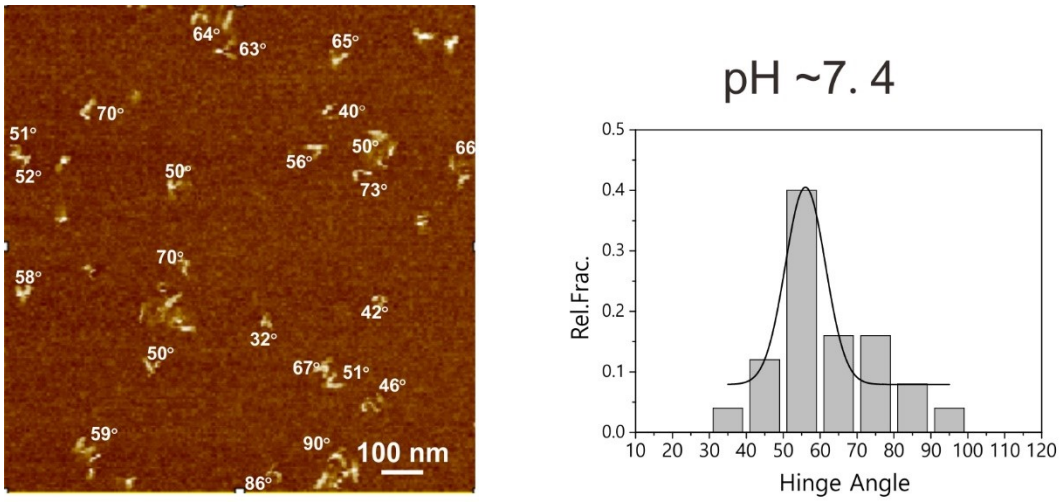

Figure S13. Hinge angle analysis for the nanocaliper in the AFM images (5 mM Tris, 1 mM EDTA, 12.5 mM $MgCl_2$, and pH ~7.4).

Figure S14. Hinge angle analysis for the nanocaliper in the AFM images (5 mM Tris, 1 mM EDTA, 12.5 mM $MgCl_2$, and pH ~5.0).

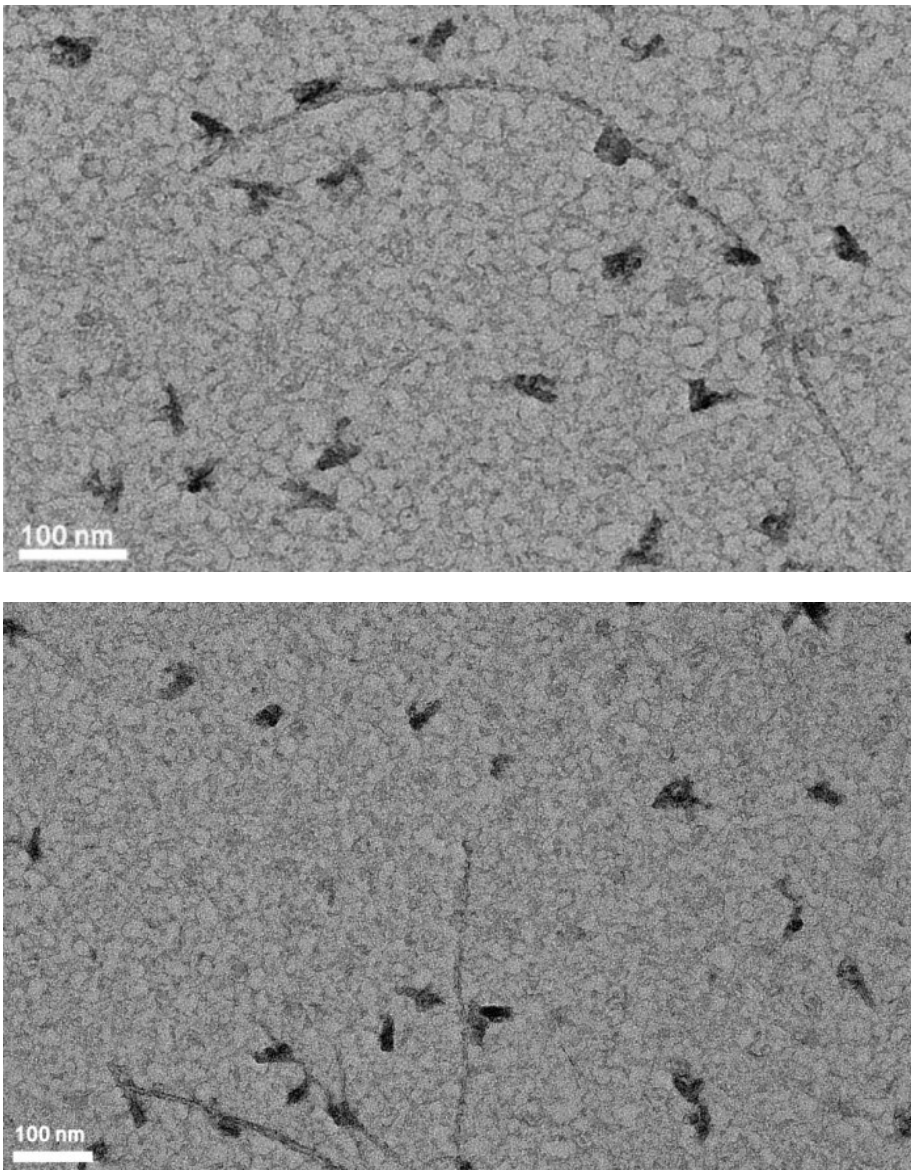

Figure S15. The TEM images of nanocalipers incubated with carbon nanotubes in Tris buffer ((5 mM Tris, 1 mM EDTA, 12.5 mM $MgCl_2$, and pH ~7.4)).

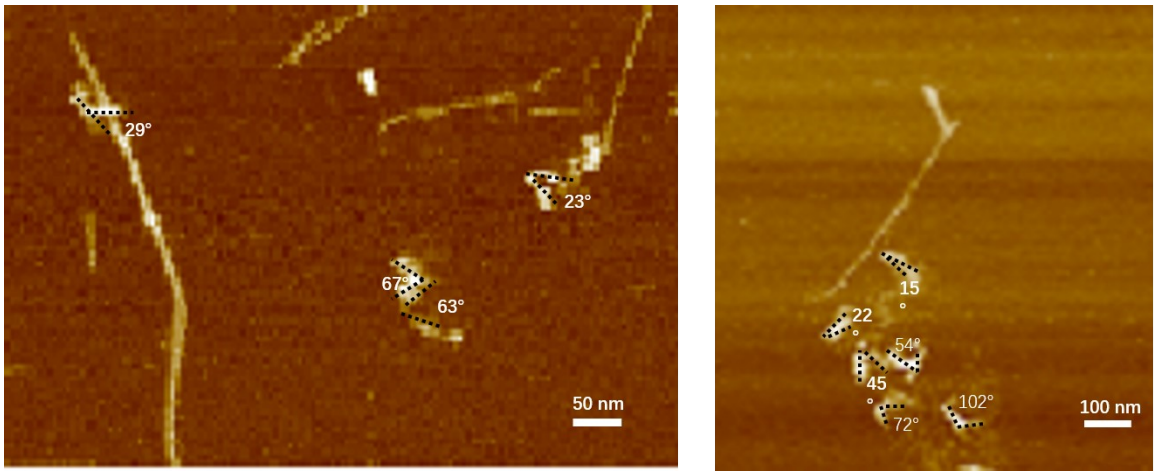

Figure S16. The AFM images of nanocalipers incubated with carbon nanotubes in Tris buffer ((5 mM Tris, 1 mM EDTA, 12.5 mM $MgCl_2$, and pH ~7.4)).

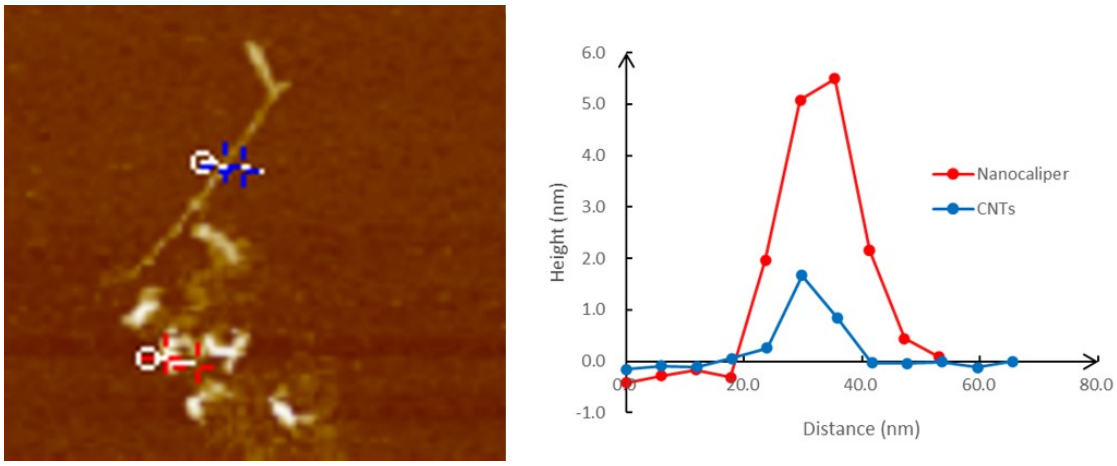

Figure S17. The detailed AFM images of nanocalipers incubated with carbon nanotubes and the height analysis of CNT and nanocaliper in the AFM images. Nanocalipers and the CNT can be distinguished by the height and they were ~1.8nm and ~5.5nm, respectively.