Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information

Control of the Stepwise Assembly-Disassembly of DNA Origami Nanoclusters by pH Stimuli-Responsive DNA Triplexes

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MATERIALS

All chemicals including agarose, magnesium acetate, acetic acid, sodium hydroxyl, hydrogen chloride, were purchased from Sigma and used as received without further purification. All chemically synthesized DNA strands were purchased from Integrated DNA Technologies, Inc. (www.Idtdna.com). The unmodified staple DNA strands were ordered in a 96-well plate format, suspended in ultrapure water without purification. All modified strands were purified with PAGE. A circular single-stranded M13mp18 DNA genome was ordered from Bayou Biolabs. The DNA origami purification column (100kDa MWCO centrifuge filter) was purchased from Pall, Inc.

EXPERIMENTAL METHODS

The formation of cross-shaped DNA origami unit

The cross-shaped DNA origami was assembled by a slow cooling method. Specifically, M13mp18 viral DNA and all of the staple strands were mixed together at a ratio of 1:5, in a 1×TAE buffer solution containing 40 mM Tris-HCl, 20 mM of acetic acid, 2 mM of EDTA, and 11.5 mM of magnesium acetate. The mixture was slowly cooled from 90℃ to 15℃ over 12h in a thermocycler (BIO-RAD). The final concentration of M13mp18 DNA in the solution was 20 nM. The DNA origami was then purified to remove excess staple strands, using 100kDa MWCO centrifuge filters.

The assembly-disassembly of DNA origami dimer

The assembly/disassembly of DNA origami dimer (A1/A2) between pH = 5.0 and pH = 7.5: the prepared DNA origami monomers were mixed at a molar ratio of 1:1 in a 1×TAE buffer solution (pH = 5.0) containing 10 mM of magnesium acetate. Then, the mixture was adjusted to pH=7.5, and annealed from 38℃ to 25℃ at a rate of 2.1℃/h to form the corresponding DNA origami dimers. In order to disassembly of DNA origami dimer, the pH value of the dimer solution was adjusted from 7.5 to 5 by column exchange in a 1×TAE buffer with 10 mM Mg^{2+}, then incubated at room temperature for 6h. The assembled/disassembled DNA dimer was directly used for AFM images, and gel electrophoretic characterization without further purification. The association/dissociation of the DNA origami dimer was performed by further buffer exchange following the same incubation step as the aforementioned

procedure. The same process was followed for assembly/disassembly of the dimer (A2/A3), except the pH values changed to between pH = 7.5 and pH = 9.0.

The assembly-disassembly of DNA origami trimer

A. Stepwise upstream assembly process of DNA origami trimer (pH: 5.0 -7.5 – 9.0)

1. The prepared three DNA origami monomers (A1, A2, and A3) were mixed at a molar ratio of 1:1:1 in a 1×TAE buffer solution (pH = 5.0) containing 10 mM of magnesium acetate.

2. Then the mixture was adjusted to pH=7.5, and annealed from 35℃ to 25℃, at a rate of 0.8/h to form DNA origami dimers by activating the sticky end interaction in DNA triplex set T1.

3. Next, the centrifuge column was used to exchange the buffer solution to pH = 9.0. Then the mixture was re-annealed from 30℃ to 25℃ at a rate of 0.8℃/h to form a DNA origami trimer through activating the sticky end interaction in DNA triplex set T2 (73%TAT). The assembled DNA origami trimers were then directly imaged by AFM without further purification.

B. Stepwise downstream disassembly process of DNA origami trimer (pH: 9.0 - 7.5 – 5.0)

1. The prepared DNA origami monomers were mixed at a molar ratio of 1:1:1 in a 1×TAE buffer solution (pH = 9) containing 10 mM of magnesium acetate.

2. Then the mixture was annealed from 35℃ to 25℃ at a rate of 0.8℃/h to form DNA origami trimer through activating the sticky end interaction in both DNA triplexes T1 (20%TAT) and T2 (73%TAT). The dissociation of the trimers was allowed to proceed for 6 h at room temperature after changing the pH value to 7.5 through deactivating the sticky end in DNA triplex set T2 (73%TAT) only.

3. With further adjustment of the pH value to 5 with another 6h incubation at room temperature, the trimers could be totally separated into monomers with the deactivation of sticky end interaction in both DNA triplex T1 (20%TAT), and T2 (73%TAT).

The formation of 9-tile DNA origami

The prepared DNA origami monomers (A4, A5, and A6) were mixed at a molar ratio of 1:4:4 in a 1×TAE buffer solution (pH = 5.0) containing 10 mM of magnesium acetate. Then the mixture was adjusted to pH=7.5, annealed from 50℃ to 25℃ at a rate of 2℃/h by selectively bridging titles A4 and

A5 together to form 5-tile DNA origami through DNA triplex set T1, while keeping DNA title A6 as a monomer. Next, the centrifuge column was used again to exchange the buffer solution to pH = 9.0. The mixture was reannealed from 45℃ to 25℃ at a rate of 3.3℃/h to form a 9-tile DNA origami by bridging title A6 to preformed 5 title units through DNA triplex set T2.

The disassembly of 9-tile DNA origami

First, the prepared DNA origami monomers were mixed at a molar ratio of 1:4:4 in a 1×TAE buffer solution (pH = 9) containing 10 mM of magnesium acetate. Then the mixture was annealed from 45℃ to 25℃ at a rate of 1.2℃/h to form 9-tile DNA origami. Second, adjusting the pH value to 7.5, and incubating at room temperature for 6h, 9-tile DNA origami units could be dissociated to 5-tile DNA origami units, and monomer units through deactivating the sticky end cohesion in 73%TAT triplex (T2). By further adjusting the pH value to 5.0, and incubating another 6h at room temperature, the 5-tile DNA origami disassembled to monomers through deactivating the sticky end in 20%TAT DNA triplexes (T1).

AFM imaging

The AFM images of DNA origami were obtained through spotting each sample (3 μl) onto freshly cleaved muscovite mica (Ted Pella, Inc.) for 15 s. After the fixation of the targeted structure of DNA origami on mica surface, doubly distilled H_2O (20-30 ul) was placed quickly on the mica to remove the buffer salts, the drop was wicked off, and the sample was dried with compressed air. Atomic force imaging was done by utilizing Nanoscope III (Digital Instruments) tapping in air; with ultra-sharp 14 series (NSC 14) tips that had been purchased from NANOANDMORE.

Agarose Gel Electrophoresis

For the agarose gel under pH =5.0 and 7.5; the samples were loaded into 0.8% agarose gel with adjusted pH values that contained 5 mM Mg $(CH_3COO)_2$ in a 1×TAE buffer solution under 55V at room temperature. For the agarose gel under pH =9, the concentration of $Mg(CH_3COO)_2$ was changed to 2 mM. The gel was stained with ethidium bromide for visualization.

Dynamic light scattering

The size distribution of DNA origami nanostructures was measured with a DLS analyzer (Zetasizer ZS90, Malvern, UK). The DNA origami was diluted to 10 nM by a 1× TAE buffer with 10 mM Mg^{2+}, and injected into a 70 uL plastic cuvette to measure the size distributions. This procedure was repeated three times.

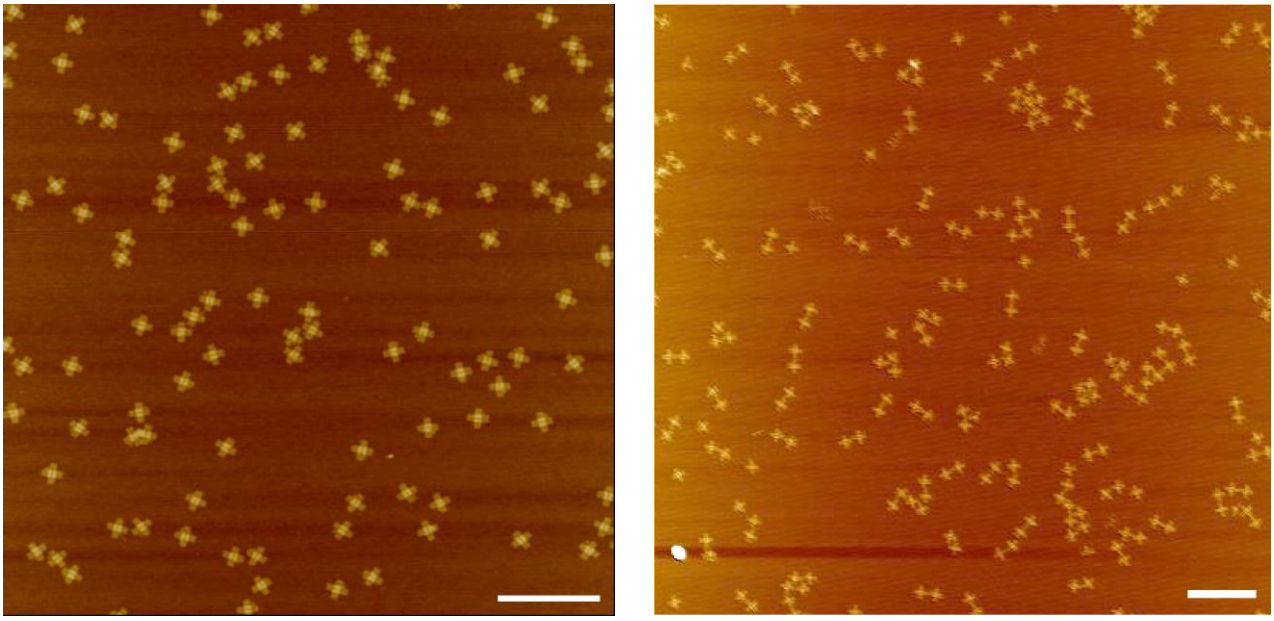


Figure S1: The representative AFM images of DNA origami monomer (A1) (left image) and dimer (A1/A2) (right image) without purification. The calculated yield of dimer is ~87% based on AFM results. Scale bar: 500 nm.

The equation below was used to calculate the yield of dimer.

$$\%yield = \frac{Numbers\ of\ dimers\ x2}{total\ numbers\ of\ DNA\ origami}$$

pH values		Monomer (A1, A2)	Dimer (A1/A2)	Total origamis
5.0	Origamis Counted	234	24	258
	Yield (%)	91	9	
7.5	Origamis Counted	44	286	330
	Yield (%)	13	87	

Table S1: The statistical analysis of AFM images of DNA origami monomer and dimer structures generated at pH 5.0 and pH 7.5.

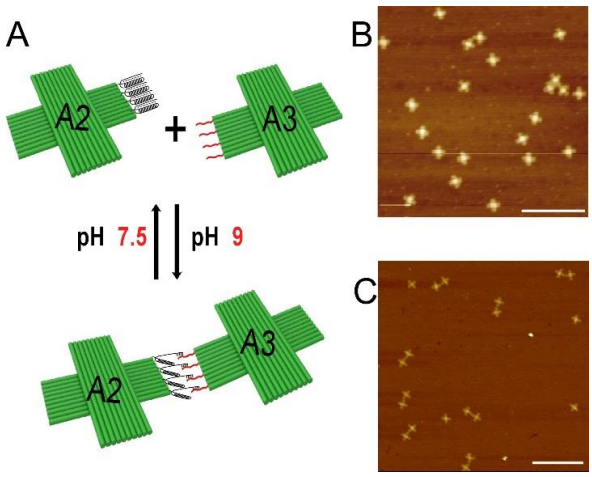


Figure S2. DNA triplexes (73% T-A·T) -driven assembly/disassembly of DNA origami dimer A2/A3. (A) Schematic drawing of the pH-stimulated cyclic assembly of DNA origami dimers through duplex-triplex transition of DNA triplexes between pH 7.5 and 9. (B) AFM images of DNA origami monomer at pH=7.5 and dimer at pH=9.0 (C). Scale bars: 500 nm.

pH values		Monomer (A2, A3)	Dimer (A2/A3)	Total origamis
7.5	Origamis Counted	144	18	162
	Yield (%)	89	11	
9.0	Origamis Counted	27	162	189
	Yield (%)	14	86	

Table S2: The statistical analysis of AFM images of DNA origami monomer and dimer structures generated at pH 7.5 and pH 9.0.

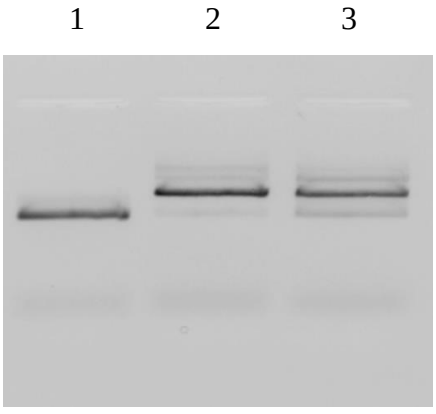


Figure S3: Gel electrophoresis used to compare the yield of dimer at pH 7.5 with and without thermal annealing process. Lane 1: DNA origami monomer control. Lane 2: Self-assembled dimer A1/A2 annealed from 38℃ to 25℃ over 6h. Lane 3: Assembled dimer A1/A2 incubated at room temperature. The dimer yield of lane 2 and lane 3 are 89%, and 75%, respectively.

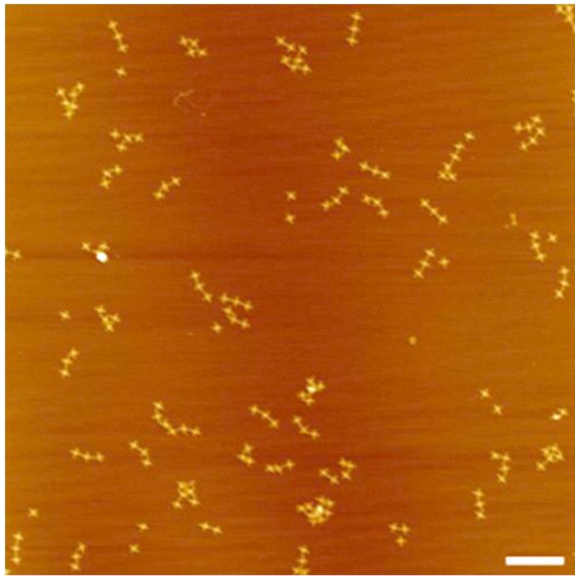


Figure S4: A representative AFM image of DNA origami timer (A1/A2/A3) without purification. The calculated trimer yield was ~70% based on AFM results. Scale bar: 500nm.

pH values		Monomer (A1, A2, A3)	Dimer (A1/A2, A3)	Trimer (A1/A2/A3)	Total origami
5.0	Origamis Counted	158	12	0	170
	Yield (%)	93	7	0	
7.5	Origamis Counted	68	90	15	173
	Yield (%)	39	52	9	
9.0	Origamis Counted	33	22	126	181
	Yield (%)	18	12	70	

Table S3: The statistical analysis of AFM images of origami monomer, dimer and trimer structures generated at pH 5.0, pH 7.5 and pH 9.0.

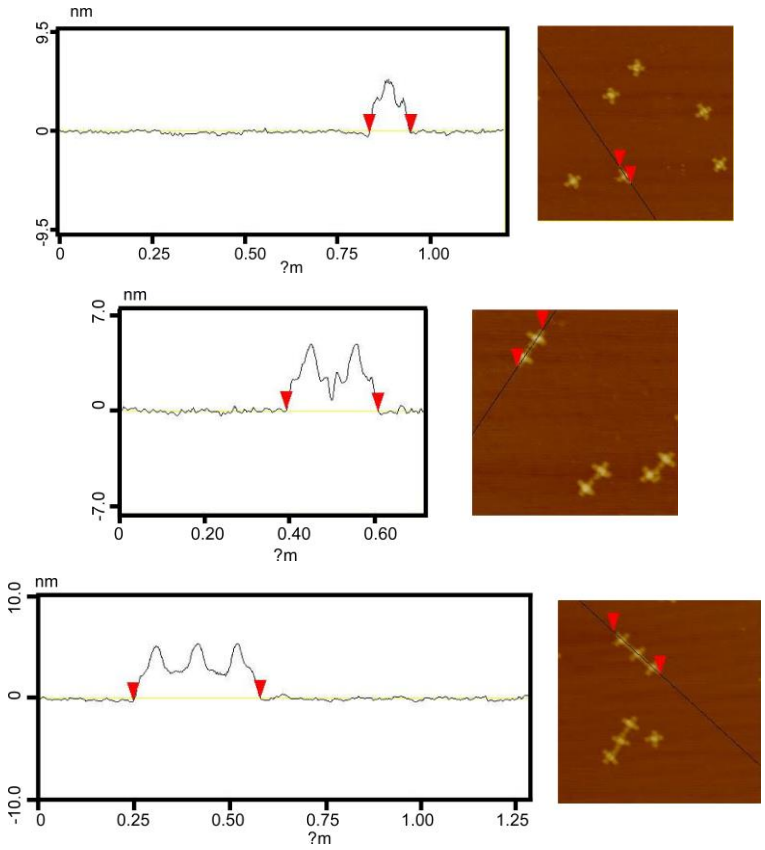


Figure S5: Cross-section analysis of self-assembled DNA origami monomer, dimer, and trimer. The size of monomer is ~ 100 nm, dimer is ~200 nm, and trimer is ~300 nm, which is consistent with design.

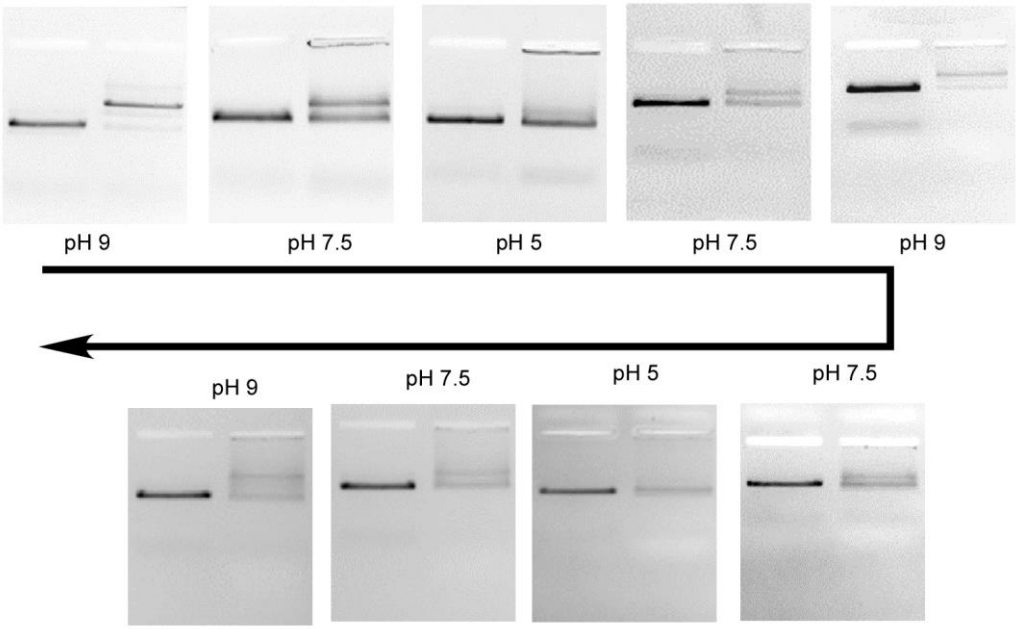


Figure S6: Gel electrophoresis to show the reversible, multistep assembly of DNA origami nanostructures driven by DNA triplexes in response to pH. For each image, the left lane and right lane represent DNA origami monomer control and sample, respectively. For each of the two working cycles, the DNA trimer (pH=9) dissociates to dimer (pH=7.5) and further to monomer (pH=5), and the process reverses when the pH increases.

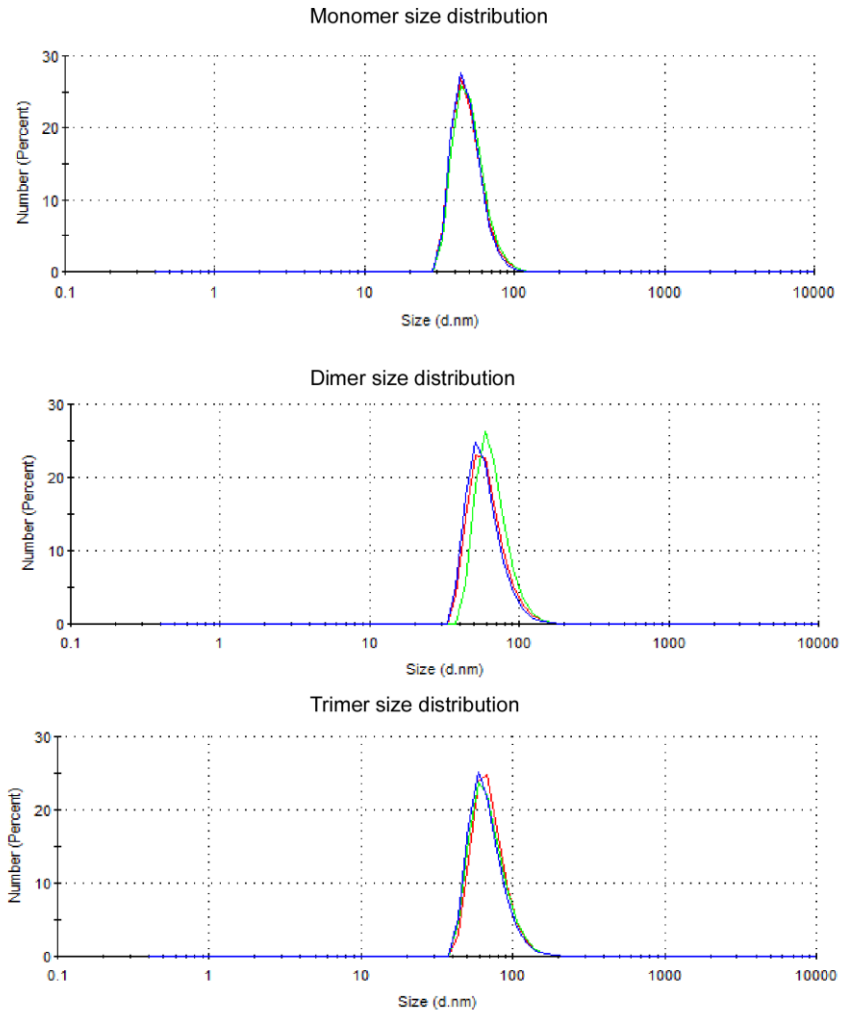


Figure S7: Hydrodynamic size distribution of the DNA origami nanostructures from DLS measurements. The three different colored (red, green, and blue) lines represent the three runs. The average size of monomer, dimer, and trimer is 49.43 ± 0.90 nm, 62.73 ± 3.81 nm, and 70.03 ± 1.39 nm, respectively.

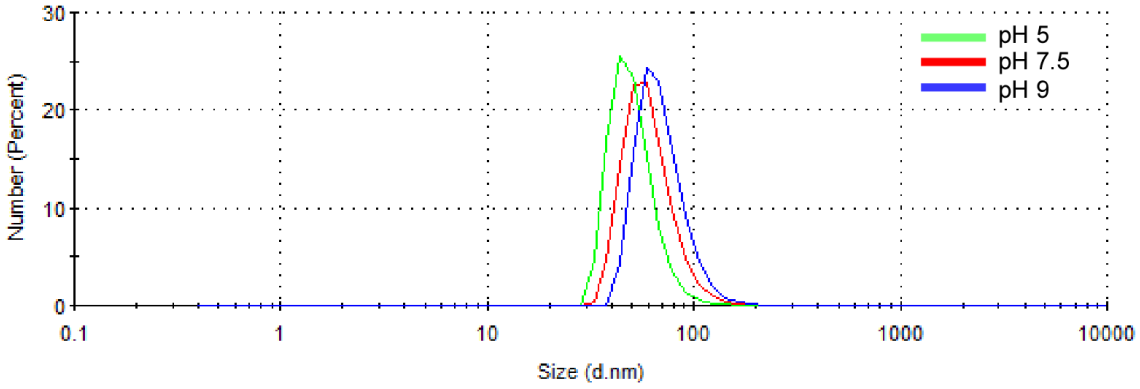


Figure S8: DLS measurements to show the size variations of DNA origami nanostructures induced by pH titration. The size distribution shifted to the left when the pH changed from pH 9, pH 7.5, to pH 5, corresponding to the sizes of DNA origami trimers, dimers, and monomers, respectively, which is consistent with our design.

A B

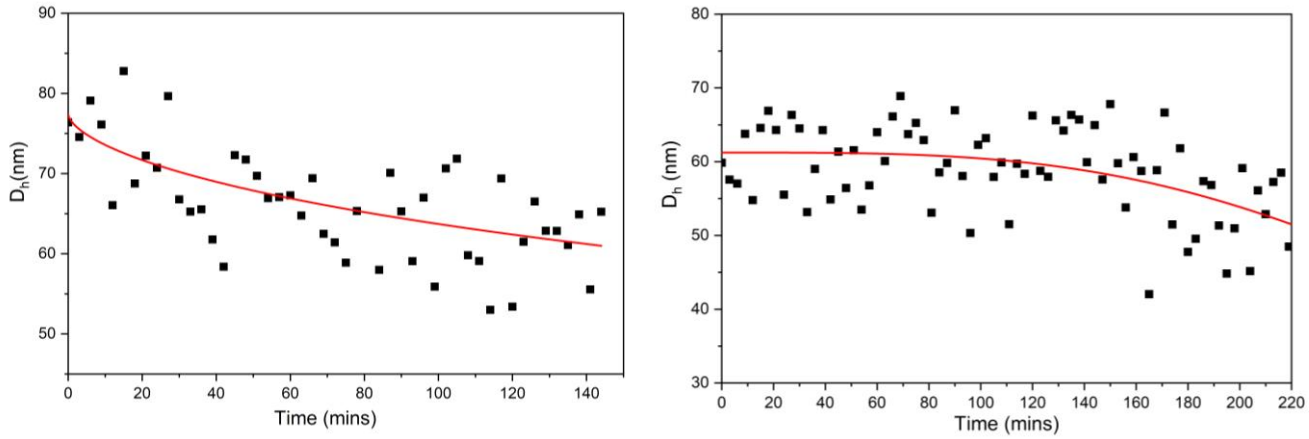


Figure S9: Time-dependent DLS study of the dynamic dissociation of DNA origami nanostructures driven by DNA triplexes. A) The DNA origami trimers dissociated to dimers when the pH was changed from 9 to 7.5. B) The DNA origami dimers dissociated to monomers when the pH was changed from 7.5 to 5.

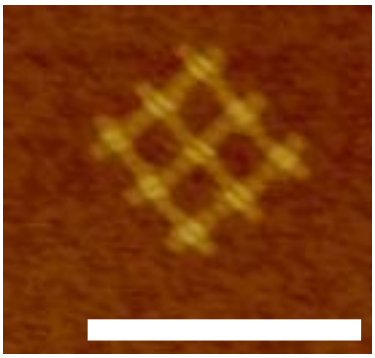


Figure S10: AFM image of assembled 9-tile DNA origami. Rotation of the origami units exactly followed our theoretical design. Scale bar: 500nm.

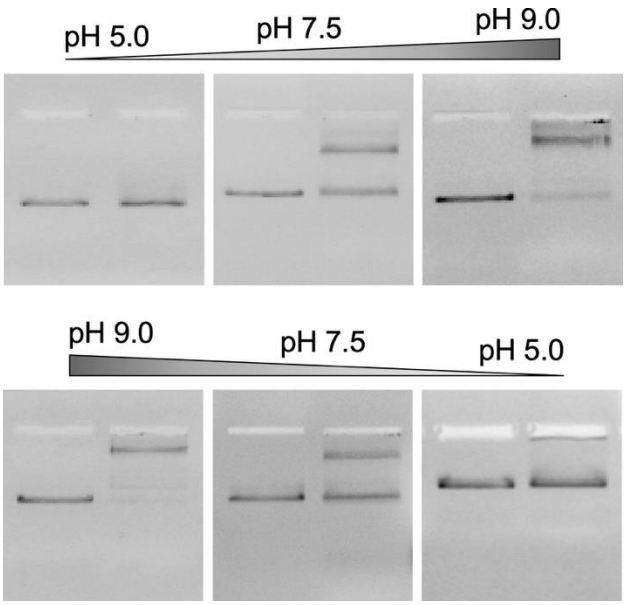
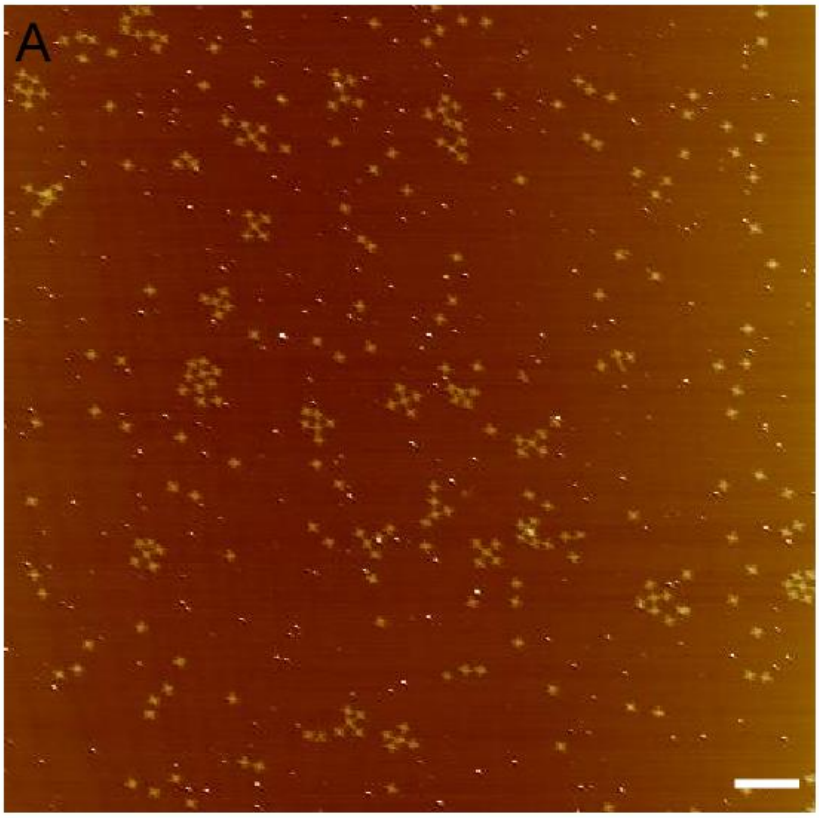


Figure S11. Stepwise and reversible assembly of DNA origami 9-tile clusters demonstrated by agarose gel electrophoresis. Left lane of each image is DNA origami monomer, used as reference control, to show the bands mobility of targeted 5-titles and 9-titles. The yield of the 5-tile structure is 66.9%, while the yield of 9-tile is 72.8% based on gel electrophoresis.



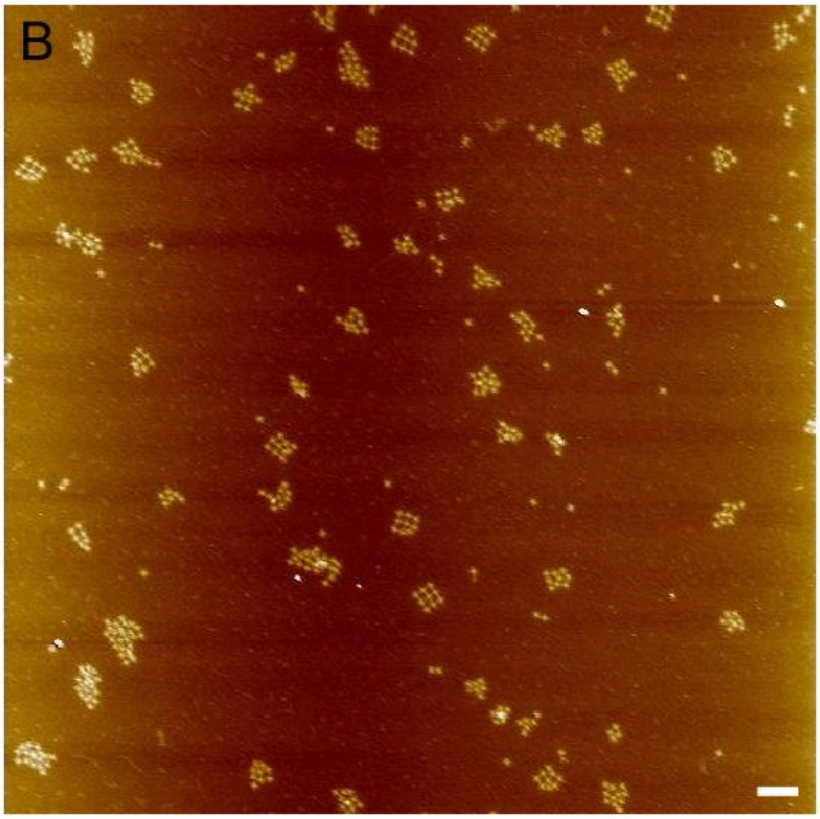


Figure S12: AFM images of unpurified 5-tile and 9-tile DNA origami. A) A representative 5-tile DNA origami. B) A representative 9-tile DNA origami. Aggregation and partial formation of DNA origami coexist with targeted structures. Scale bar: 500nm

DNA Sequences used in this design:

RC-M1 AGCTAATGCAGAACGCGCCTGTTTTAATATCC
RC-M2 CATCCTAATTTGAAGCCTTAAATCTTTTATCC
RC-M3 TGAATCTTGAGAGATAACCCACAAAACAATGA
RC-M4 AATAGCAATAGATGGGCGCATCGTACCGTATC
RC-M5 GGCCTCAGCTTGCATGCCTGCAGGGAATTCGT
RC-M6 AATCATGGTGGTTTTTCTTTTCACCCGCCTGG
RC-M7 CCCTGAGAGAGTTGCAGCAAGCGGGTATTGGG
RC-M8 CGCCAGGGTCATAGCTGTTTCCTGGACGGCCA
RC-M9 GTGCCAAGGAAGATCGACATCCAGATAGGTTA
RC-M10 CGTTGGTGTAGCTATCTTACCGAATTGAGCGC
RC-M11 TAATATCAACCTTCGCTAACGAGCCCGACTTG
RC-M12 CGGGAGGTTTTACGAGCATGTAGAACATGTTC
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RC-M15 AGAGCCTACAAAGTCAGAGGGTAAGCCCTTTT
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RC-M165 GACAAAAGGAAACCATCGATAGCATTTGCCAT
RC-M166 AAAGGTGGCAACATATAAAAGAAACACAATCA
RC-M167 ATCAGTAGTTCATATGGTTTACCAACATACAT
RC-M168 TGGATCTTAGCCCCCTTATTAGCGGCACCGTA
RC-M169 ATAAGTATTTTTGATGATACAGGACAAACGAA
RC-M170 ACTTTCAACTCAGAACCGCCACCCGGGTTGAT
RC-M171 ACAGCATCGTTGAAAATCTCCAAAGCTAAACA
RC-M172 GAAGTTTCCATTAAACGGGTAAAAAGCGAAAG
RC-M173 TTTTTCACGGAACGAGGGTAGCAATTCATGAG

RC-M174 CCGCCACCCAGTTTCAGCGGAGTGATAATAAT
RC-M175 TACATGGCAGCCCGGAATAGGTGTCCTCAGAA
RC-M176 TCGGTCATCATTAAAGCCAGAATGAAGCGTCA
RC-M177 ATAGAAAACGACAGAATCAAGTTTCGGCATTT
RC-M27-AS CCATATTAATTAGACGGGAGAATTACAAAGTTACC
RC-M29-AS AAGCGCCAATTAAGTTGGGTAACGAACATACG
RC-M37-AS GATTTTTTACAGAGAGAATAACATAAAAACAG
RC-M38-AS TTGGGAAGCAGCTGGCTTAAAGCTAGCTATTTTTGAGAGAT
RC-M49-AS ACCTGAGCAGAGGCGAATTATTCAGAAAATAG
RC-M50-AS AGAAGTATAATAGATAATACATTTCTCTTCGC
RC-M57-AS CAAGAAAAATTGCTTTGAATACCAAGTTACAA
RC-M58-AS CTCGTATTGGTGCACTAACAACTAGAACGAAC
RC-M68-AS TGATTTGATACATCGGGAGAAACACAACGGAG
RC-M70-AS ATTTTAAAGGAATTGAGGAAGGTTTGAGGCGG
RC-M109-AS AAACGAGACGACGATAAAAACCAAACTAACGG
RC-M111-AS TGCGGGAGGAAAAGGTGGCATCAAACTAAAGT
RC-M121-AS GAATCCCCTGCAAAAGAAGTTTTGGTTGGGAA
RC-M131-AS CCAATACTTAAAATGTTTAGACTGGTAGCATT
RC-M133-AS ATAAAGCCGCAAAGAATTAGCAAACCACCACC
RC-M141-AS TCACCAGTAGCCCTCATATGATGAAAGACTACC
RC-M143-AS CCCTCAGACGCCACCAGAACCACCATGCCCCC
RC-M122-AS GTACCAAAAGCATTAACATCCAATGGTGCTGTAGCTCAACATGTTT
RC-M151-AS TAGGAACCTTGTCGTCTTTCCAGACGGTTTATCAGCTTGCGGCTTGCA
RC-M152-AS CACCACCGGCATTGACAGGAGGTTGCCTTGAGTAACATAATTTAGGCAG

Modified DNA Sequences used for the formation of DNA origami trimer:

20%TAT Triplex
Triplex-A1R1 GTGTGATAAATAAGGCTTTTT
Triplex-A1R2 ATAACCTTGCTTCTGTTTTTT
Triplex-A1R4 AGCGGAATTATCATCATTTTT
Triplex-A1R5 ATCTAAAGCATCACCTTTTTT
Triplex-A1R1G
CTCATGCCTCCCCTCCCCTCCGTTTCCCTCCCCTCCCCTCCATTTGGAGGGGAGGGGAGGTTTTTTTT
GTTAAATAAGAATAAA
Triplex-A1R2G
CTCATGCCTCCCCTCCCCTCCGTTTCCCTCCCCTCCCCTCCATTTGGAGGGGAGGGGAGGTTTTTTTT
AAATCGTCGCTATTAA
Triplex-A1R4G
CTCATGCCTCCCCTCCCCTCCGTTTCCCTCCCCTCCCCTCCATTTGGAGGGGAGGGGAGGTTTTTTTT
TATTCCTGATTATCAG
Triplex-A1R5G
CTCATGCCTCCCCTCCCCTCCGTTTCCCTCCCCTCCCCTCCATTTGGAGGGGAGGGGAGGTTTTTTTT
TGCTGAACCTCAAATA
Blunt RE3 TTTTAAATAAAGAAATTGCGTTAGCACGTAAAACAGTTTT
Blunt RE6 TTTTACATTGGCAGATTCACCTGAAATGGATTATTTTTTT

Triplex-A2L1 GGGAGGCATGAGTTTTTTTTTCCTGAACAAGAAAAAATCAACAATAGATAAGTTTTT
Triplex-A2L2 GGGAGGCATGAGTTTTTTTTTTGCACCCAGCTACAAAAGATTAGTTGCTATTTTTTT
Triplex-A2L4 GGGAGGCATGAGTTTTTTTTGTTTGAGGGGACGACGAACCGTGCATCTGCCATTTTT
Triplex-A2L5 GGGAGGCATGAGTTTTTTTTCCCGGGTACCGAGGTCTCGACTCTAGAGGATCTTTTT
Blunt LE3 TTTTAATAATAAGAGCAAGAGAATTGAGTTAAGCCCTTTT
Blunt LE6 TTTTAGCTGATTGCCCTTCACAGTGAGACGGGCAACTTTT

73%TAT Triplex
Triplex-A1R1 GTGTGATAAATAAGGCTTTTT
Triplex-A1R2 ATAACCTTGCTTCTGTTTTTT
Triplex-A1R4 AGCGGAATTATCATCATTTTT
Triplex-A1R5 ATCTAAAGCATCACCTTTTTT
Triplex-A2R1
TCGTATTTCTTCTTCTTTCTTGTTTCTTCTTTCTTCTTCTTATTTAAGAAGAAGAAAGAATTTTTTTTGT
TAAATAAGAATAAA

Triplex-A2R2
TCGTATTTCTTCTTCTTTCTTGTTTCTTCTTTCTTCTTCTTATTTAAGAAGAAGAAAGAATTTTTTTTA
AATCGTCGCTATTAA
Triplex-A2R4
TCGTATTTCTTCTTCTTTCTTGTTTCTTCTTTCTTCTTCTTATTTAAGAAGAAGAAAGAATTTTTTTTTA
TTCCTGATTATCAG
Triplex-A2R5
TCGTATTTCTTCTTCTTTCTTGTTTCTTCTTTCTTCTTCTTATTTAAGAAGAAGAAAGAATTTTTTTTTG
CTGAACCTCAAATA
Blunt RE3 TTTTAAATAAAGAAATTGCGTTAGCACGTAAAACAGTTTT
Blunt RE6 TTTTACATTGGCAGATTCACCTGAAATGGATTATTTTTTT

Triplex-A3L1 GAAGAAATACGATTTTTTTTTCCTGAACAAGAAAAAATCAACAATAGATAAGTTTTT
Triplex-A3L2 GAAGAAATACGATTTTTTTTTTGCACCCAGCTACAAAAGATTAGTTGCTATTTTTTT
Triplex-A3L4 GAAGAAATACGATTTTTTTTGTTTGAGGGGACGACGAACCGTGCATCTGCCATTTTT
Triplex-A3L5 GAAGAAATACGATTTTTTTTCCCGGGTACCGAGGTCTCGACTCTAGAGGATCTTTTT
Blunt LE3 TTTTAATAATAAGAGCAAGAGAATTGAGTTAAGCCCTTTT
Blunt LE6 TTTTAGCTGATTGCCCTTCACAGTGAGACGGGCAACTTTT

Modified DNA Sequences used for the formation of 9-tile origami:

pH-A4-Left-linearSE1
GGGAGGCATGAGTTTTTTTTTCCTGAACAAGAAAAAATCAACAATAGATAAGTTTTT
pH- A4-Left-linearSE2
GGGAGGTCTCAATTTTTTTTTTGCACCCAGCTACAAAAGATTAGTTGCTATTTTTTT
pH- A4-Left-linearSE4
GGGAGGATACATTTTTTTTTGTTTGAGGGGACGACGAACCGTGCATCTGCCATTTTT
pH- A4-Left-linearSE5
GGGAGGTAGTCATTTTTTTTCCCGGGTACCGAGGTCTCGACTCTAGAGGATCTTTTT

pH-A4-Right-linearSE6
GGGAGGCATGAGTTTTTTTTACATTGGCAGATTCACCTGAAATGGATTATTTTTTT
pH-A4-Right-linearSE5
GGGAGGTCTCAATTTTTTTTTGCTGAACCTCAAATAATCTAAAGCATCACCTTTTTT
pH-A4-Right-linearSE3
GGGAGGATACATTTTTTTTTAAATAAAGAAATTGCGTTAGCACGTAAAACAGTTTTT
pH-A4-Right-linearSE2
GGGAGGTAGTCATTTTTTTTAAATCGTCGCTATTAAATAACCTTGCTTCTGTTTTTT

pH-A4-Down-linearSE1
GGGAGGCATGAGTTTTTTTTCGTTAATATTTTGTTAATATTTAAATTGTAAATTTTTTTT
pH-A4-Down-linearSE2
GGGAGGTCTCAATTTTTTTTTGAGTAATGTGTAGGTTTTTAAATGCAATGCCTTTTT
pH-A4-Down-linearSE4
GGGAGGATACATTTTTTTTTATCAAAAAGATTAAGAAAGCAAAGCGGATTGCTTTTT
pH-A4-Down-linearSE5
GGGAGGTAGTCATTTTTTTTATAACGCCAAAAGGAACAACTAATGCAGATACTTTTT

pH-A4-Top-linearSE6
GGGAGGCATGAGTTTTTTTTGAGGACTAAAGACTTTCGGCTACAGAGGCTTTTTTTT
pH-A4-Top-linearSE5
GGGAGGTCTCAATTTTTTTTACTAAAGGAATTGCGAAGAATAGAAAGGAACATTTTT
pH-A4-Top-linearSE3
GGGAGGATACATTTTTTTTTAATTTACCGTTCCAGTGAAAGCGCAGTCTCTGTTTTT
pH-A4-Top-linearSE2
GGGAGGTAGTCATTTTTTTTTGTAGCGCGTTTTCATGCCTTTAGCGTCAGACTTTTT

pH-A5-Right-Triplex SE1
CTCATGCCTCCCCTCCCCTCCGTTTCCCTCCCCTCCCCTCCATTTGGAGGGGAGGGGAGGTTTTTTTT
GTTAAATAAGAATAAA
pH-A5-Right-Triplex SE2
TTGAGACCTCCCCTCCCCTCCGTTTCCCTCCCCTCCCCTCCATTTGGAGGGGAGGGGAGGTTTTTTAA
ATCGTCGCTATTAAATAACCTT

pH-A5-Right-Triplex SE4
ATGTATCCTCCCCTCCCCTCCGTTTCCCTCCCCTCCCCTCCATTTGGAGGGGAGGGGAGGTTTTTTTA
TTCCTGATTATCAGAGCGGAAT
pH-A5-Right-Triplex SE5
TGACTACCTCCCCTCCCCTCCGTTTCCCTCCCCTCCCCTCCATTTGGAGGGGAGGGGAGGTTTTTTTG
CTGAACCTCAAATAATCTAAAG

pH-A5-TOP-linearSE2
GAAGAAACGATTTTTTTTTTTGTAGCGCGTTTTCATGCCTTTAGCGTCAGACTTTTT
pH-A5-TOP-linearSE4
GAAGAACTACCGTTTTTTTTGGTTTAGTACCGCCACATCACCGTACTCAGGATTTTT

pH-A5-Down-linearSE2
GAAGAAGATGACTTTTTTTTTGAGTAATGTGTAGGTTTTTAAATGCAATGCCTTTTT
pH-A5-Down-linearSE4
GAAGAACCTCGATTTTTTTTATCAAAAAGATTAAGAAAGCAAAGCGGATTGCTTTTT

pH-A6-TOP-TriplexS2
GTCATCTTCTTCTTCTTTCTTGTTTCTTCTTTCTTCTTCTTATTTAAGAAGAAGAAAGAATTTTTTTGT
AGCGCGTTTTCATGCCTTTAG
pH-A6-TOP-TriplexS4
TCGAGGTTCTTCTTCTTTCTTGTTTCTTCTTTCTTCTTCTTATTTAAGAAGAAGAAAGAATTTTTTGGT
TTAGTACCGCCACATCACCGT

pH-A6-Right-TriplexS3
CGGTAGTTCTTCTTCTTTCTTGTTTCTTCTTTCTTCTTCTTATTTAAGAAGAAGAAAGAATTTTTTAAA
TAAAGAAATTGCGTTAGCACG
pH-A6-Right-TriplexS5
AATCGTTTCTTCTTCTTTCTTGTTTCTTCTTTCTTCTTCTTATTTAAGAAGAAGAAAGAATTTTTTTGC
TGAACCTCAAATAATCTAAAG