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Supporting Information for

Salt-Enabled Visual Detection of DNA

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1. Materials, facilities, and measurements

Hydrogen peroxide (30 wt%, AR grade) was purchased from Yonghua Chemical Technology (Jiangsu) Co. Ltd. Concentrated sulfuric acid (95-98%), hydrochloric acid (36-38 wt%), ethylenediaminetetraacetic acid disodium salt dihydrate （EDTA, 99.0%), boric acid (99.5%), and ammonia solution (25-28 wt%) were purchased from Nanjing Chemical Reagent Co. Ltd. 3-(2-Aminoethylamino)propyltrimethoxysilane (EDAS, 96%), hexanoic anhydride (HA, 98%), sodium dihydrogen phosphate dehydrate (99%), sodium hydrogen phosphate (anhydrous, 99.0%), and sodium chloride (99%) were purchased from Alfa Aesar. Ethanol (99.7%) was from Shanghai Titanchem Co. Ltd. Succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC, 98% HPLC) was purchased from Meyer Chemical Technology Co. Ltd. Glacial acetic acid (99.8%) was from Acros Organics. 1-Hexanethiol (HT, 96%) was from Adamas-beta. Tris(hydroxymethyl)aminomethane (Tris, 99.8%) was purchased from Nanjing Biosky Sci. & Tech. Co. Ltd. $NaNO_3$ (96%) was from Xilong Chemical Co. Ltd. Magnesium chloride hexahydrate (99.0%) was purchased from Sigma. Sodium dodecyl sulfate (CP, SDS), dimethyl sulfoxide (DMSO, 99.0%), pyridine (99.5%), and *N,N*'-dimethylformamide (DMF, 99.5%) were obtained from Sinopharm Chemical Reagent Co. Ltd. DMSO, pyridine, and DMF were distilled over CaH_2 prior to use, and other reagents were used as received. *Taq* DNA ligase was from New England Biolabs. φ29 DNA polymerase was from Thermo Scientific. *E coli.* Exonuclease I, *E coli.* exonuclease III, dNTPs (10mM), DL2000 DNA marker, and 20 bp DNA ladder marker were purchased from Takara Biotechnology (Dalian) Co. Ltd. Diethylpyrocarbonate (DEPC) and ethidium bromide were purchased from Sangon Biotech (Shanghai) Co. Ltd. Freeze 'N Squeeze column was purchased from Bio-Rad Laboratories, Inc. Nano-pure water (18.2 MΩ•cm), purified by Sartorius Arium 611 system, was used throughout the experiment.

All the DNA molecules (Tables S1 and S3-S5) were custom-synthesized by Sangon Biotech (Shanghai) Co. Ltd. RNA (Table S2) was custom-synthesized by Takara Biotechnology (Dalian) Co. Ltd.

Ligase chain reaction (LCR) and the preparation of self-assembled DNA nanostructure were performed on a GeneAmp PCR system 9700 from Applied Biosystems. Contact angle measurement was performed on a KSV CAM200 optical contact angle meter. Gel electrophoresis was performed on a Bio-Rad system using 2% agarose gel or 20% polyacrylamide. Photographs were taken using Canon DIGITAL IXUS 950IS digital camera.

2. DNA and RNA sequence information

Tables S1. DNA symbol and sequence information

Description of DNA	Sequence[a]
Capture1	5’-ATTTAACAATAATCCTTTTTTTTTT-SH-3’
DNA1	5’-GGATTATTGTTAAATATTGATAAGGAT-3’
Probe1	5’-TTTTTTTTTTATCCTTATCAAT-3’
Probe1A	5’-ATCCTTATCAAT-3’
DNA1-SBM1	5’-GGATTATCGTTAAATATTGATAAGGAT-3’
DNA1-SBM2	5’-GGATTATTATTAAATATTGATAAGGAT-3’
DNA1-SBM3	5’-GGATTATTCGTTAAATATTGATAAGGAT-3’
DNA1-SBM4	5’-GGATTAT□GTTAAATATTGATAAGGAT-3’
Capture2	5’-TATCATACTAACATATTTTTTTTTT-SH-3’
DNA2	5’-TATGTTAGTATGATATAGGAATAGTTA-3’
Probe2	5’-TTTTTTTTTTTAACTATTCCTA-3’
RCA1	5’-HS-AAAAAAAAAATCATCGAGGAAGTGGCAG TC-3’
PDNA1	5’-(P)-TTAACAATAATGGTCTGCCCTGACTGCCAC TTCCTCGATGATCCTCGATGTCCTTATCAATAT-3’
AMPDNA1A	5’-ATTATTGTTAA-3’
AMPDNA1B	5’-(P)-ATATTGATAAGG-3’

[a] (P) denotes the presence of a phosphate group.

Tables S2. RNA symbol and sequence information

Description of RNA	Sequence
RNA1	5’-GGAUUAUUGUUAAAUAUUGAUAAGGAU-3’

Tables S3. DNA sequences for making **Cuboid1** and **Cuboid2**

DNA strand	Sequence (5'→3')
1	CATAATACTTTTTTTTTTTTTTTGGCTAGTAGATTGTTTGGAGTCAG
2	GCCCTTCCTTTTTTTTTTTTTTTGGAGACCAAAGAATGTTGAGCAGG
3	CCCGCCCCTTTTTTTTTTTTTTTGGGGACCGCGAGATATAAGACGTA
4	CTCCGGCCTTCTGATCCCCGCTGCCTGCCACCGAGCGCTGAGAACCGT
5	TACTACGTTCCCTCAACGGCTTACGCTCCGCAGTCGTGGCATTACGAT
6	CAATTCTCAATATCCATAAATACCACATCCCGGCTGAGCCGAGACGTG
7	TGATTCCGCCACAAACGGCTGCAAGTAGCAAGATGCTGAGTGCCGTAT
8	TTCACAACCTAAGACCGTAGACGTTAGGCGAAAAGTCGAAAAAAATGA
9	TAAAACCTCGGGCCCCGGGCAGCTGGCGGCGAACGGGAGTCCGGCGAT
10	GCGGCCCACAATTTCCAGCATTCCTTTTTTTTTTTTTTTGGAACTTA
11	ATTTAGCCCGGAGCCCTGTGCTCCTTTTTTTTTTTTTTTGGTATTGT
12	TTAGAACTCCGATACCCGATATCCTTTTTTTTTTTTTTTGGGTCCGG
13	CAGCCTTTCCTGTATAATGGCTAAGGGGCCAAAGGATCGTGAGGTCAT
14	TACGCCTATTCCAAATGTTCTGAGGGCCGGTGAAATGTGAGGCATAAA
15	CCTACCGACTCATAATTGGCAATAAGTTAAAAGCGGCGGGGTCTAGGA
16	TTTTTTTTCACCCAGTGCCCTATGCCCTTGATGGGGCGCTGCAGGATG
17	TTTTTTTTATACCATACAACTCATGTTTCCTACGCAGGGAGAGTAGAA
18	TTTTTTTTCCGCGGCTCCCAGCCCACTGACCCAGCTAAGCCAGGTCAG
19	CCATGCGTCCTACAGCCTGCGACCTACGACCTGTATTCAGGAACTCAG
20	CACCGCGCGCTTTATACACTTTACTTTTTTACAACGGGCTTGGGACCA
21	CCACTATTTTCGGTCACCTGAACCTTAAGTCCGCACGGGGAACACGAT

22	AAACAATCTACTAGCCGGAGTACAGGTGGCAGGCAGCGG GGATCAGAA
23	TATTGCCATACGTCTTATATCTCGCGGTCCCCGGGTTCCGC GGGATGT
24	TGTCACATCACGTCTCTGCCAGGGATTATGAGTCGGTAGG CGGTGATT
25	AGCGCCCCGTACTACATACATCCTACGGTTCTGACGGTAC TATACAGG
26	TTAATTCCCTTGCTCCGGGTGAATTGCGGGGCACCCAAG GACAGCGGA
27	ACCCCTGATTACCTGTCACCCGTAATCCGCCCGGTGTCAA CGACCCGG
28	GTCGGCACCAACATCCGGATAACGGTGACTGGTGGCTGG TCGTGATTT
29	CAATTGCCGCTAGCCCCCGAGTCCCGCACGCTGCGTGGA TTGGACGGG
30	CCCGGCCACCGGACCCGGGGGCCTGGGGCCCGAGGTTTT AGAGGTCCG
31	CTGAATACCTATGACCCGCAGCCCTAAGTTCCGCGCAAAT GTTTGTGG
32	CTCAGCATCTTGCTACTGGGCCGCAGGTCGTAGGTCGCA GGCTGTAGG
33	GCTTGCAAATCGCCGGACTCCCGTTCGCCGCCAGTTCTA AGGACTTAA
34	ACGATCCTTTGGCCCCGGCCGGAGATCAAGGGCATAGGG CACTGGGTG
35	CCTTAAACTCCTAGACCCCGCCGCTTTTAACTGAGAATTG GGGTCAGT
36	TATCACCCCTATCTCCTGGCCGAGGATTTTAGGGTCGCGG GTAGCGTG
37	CACGCCCAGCTATCCGTTACCCGGTCTTCTGGGGGCGGC AGCAATGTA
38	GCTATTGCATCGTGTTTGGAATGGGATCTGGGAACTAGGG ATCCGGTA
39	TAACCACAGTCTTTACACTAATCTCTGAGTTCCGGCTAAT GATGGCAG
40	TGTACTCCTTTTTTTT
41	TACTGCCCTTTTTTTT
42	CGGAACCCTTTTTTTT
43	GGGGCGGGAATCTAGG
44	CAGCGCTCCAAGTCCC
45	GGGCTGTGGAGCATTG
46	GCTTGTCCATCGGCCC
47	GGAATGCTGGAAATTG

48	TTTTTTTTGGTTTGTA
49	TTTTTTTTGGAAAACG
50	TTTTTTTTGGGATACT
51	AGCTGCCCAGTATCCC
52	ATTCACCCTTTTTTTTTTTTTTTGGGACTTG
53	CACAGCCCTTTTTTTTTTTTTTTTGGGCGGAT
54	AGGCCCCCTTTTTTTTTTTTTTTTGGGCCGAT
55	ACATTCTTTGGTCTCCGGGCAGTATGCGGAGC
56	TTGCAGCCTACAAACCGGAGCACAGGGCTCCG
57	ACGTCTACCGTTTTCCGGATATCGGGTATCGG
58	CCCTGGCACCTAGATTTACGGGTGACAGGTAA
59	CCTTGGGTGCCCCGCAGGACAAGCAGCGTGCG
60	CGTTATCCCAATGCTCGGGCTGCGGGTCATAG
61	TTAGCCATCTGACTCCGTAAGCCGTTGAGGGA
62	CTCAGAACCCTGCTCAGGTATTTATGGATATT
63	TTCGACTTTTCGCCTAGGCTAAATGTAAAAAA
64	CTCGGCCATCCGCTGTAGGATGTATGTAGTAC
65	ACCAGCCACCAGTCACTCAGGGGTCCAGAAGA
66	CCATTCCACGGACCTCGGACTCGGGGGCTAGC
67	TCACATTTCACCGGCCACGTAGTATAGGAAAC
68	CATTCGTCATACGGCAGTAAAGTGTATAAAGC
69	CTTGACTTTCATTTTTGGTTCAGGTGACCGAA
70	TTAACATCAATCACCGCCGGGTAACGGATAGC
71	CCGCGACCCTAAAATCGGCAATTGGATACGTA
72	CATCTCACAAATCACGAGATTAGTGTAAAGAC
73	TATGTCTCATGACCTCATGAGTTGTATGGTAT
74	CCAAGTTCTTTATGCCGGGCTGGGAGCCGCGG
75	GTCTTGGCCTGACCTGGATGTTAAGTGACGGA
76	GTACCTCGCATCCTGCATGGTTGAGTGAACGA
77	TGTGTCCACATTCAATGTGAGATGCAGGTTGC
78	ATCCGCGCTACGTATCGGCAGGAGCCAACGGT
79	TTCGCGTGCCACACGTACGCATGGCGTCGGGT
80	CAAACGCCTTGTGTCTGCGCGGTGTGTATTGA
81	CTTCCGATCGCCCGTAAATAGTGGTACGAAGT
82	CCGCTTCCTTTTTTTTTTTTTTTGTGCTTGC
83	TTGACACCTTTTTTTTTTTTTTTGGTTCGGA
84	ACGCGGCCTTTTTTTTTTTTTTTTGGTGAGGA
85	CCGCCTCCTTTTTTTTTTTTTTTTGGAGCAAG
86	TCGATCTCTTTTTTTTTTTTTTTTGGGACAAA
87	ATTTGCGCTTTTTTTTTTTTTTTTGAGGACAT
88	GCCACGACGCAAGCACGTATTATGGCGAAGAT
89	GGCTCAGCTCCGAACCGGAAGGGCTATGATAA
90	CATATCCCTCCTCACCGGAAGCGGGAGGATGG
91	CATCCGTCTTTGTCCCGGAGGCGGGTGCGAGA

92	ATTATCCTATGTCCTCGGCCGCGTGAGTTAGA
93	TAACTATTACAATACCGAGATCGAGGTCTTAG
94	CTCATTTCTTATCATAGGGATATGGAAACTAG
95	GTACCGTCATCTTCGCGGAATTAAACCGGGGT
96	AATTAGATCCGGGTCGAGGATAATGGATGTTG
97	TACTACCTCCATCCTCGACGGATGAGTATATT
98	CCACAGATTCTAACTCAATAGTTAATGTCAGA
99	ATCCACGCTCTCGCACTGGCCGGGACGAAGGC
100	GATCCCGTATCGTAATGTCGTGGCATTACGAT
101	AACCCTTTACCCCGGTAGGTAGTAGTTGAAAG
102	TTAAATATCTAGTTTCATCTAATTGGTAGGGT
103	ATTTCGTGAATATACTATCTGTGGGGCAGATG
104	CCCCGTGCGCCTTCGTGTTGTGAAGTTCGAGC
105	AGCCCGTTTCTGACATCGGAATCAACAAGTCA
106	TTATTCTTCTTTCAACACGGGATCGGGCACAG
107	TGCCGCCCACCCTACCATGTGACAAGGTTTGA
108	CGCATCTACATCTGCCATATTTAAGTTTTCAG
109	CGTCCGTACCCGTCCAAAAGGGTTGGAGATAG
110	GGACTCTAGCTCGAACCACGAAATAGAAAGCA
111	ATTAGCCGTGACTTGTGTGCCGACAATGCAGT
112	TCCCTGCGCTGTGCCCAAAGGCTGGATAGAGA
113	GCTTAGCTTCAAACCTTAGGCGTAGTGTGGTG
114	CCTCTACCCTGAAAACAAGAATAAGGTATGGG
115	CCAGCACCTGCTTTCTTACGGACGCTGACGTA
116	TTACAGCTACTGCATTTAGATGCGGGCGCGAG
117	TCGTGCCTTGGTCCCATAGAGTCCAGGGCAAG
118	CGCTACAACACCACACGGTAGAGGAAGAGAGG
119	TCAACCATTCTCTATCGGGTGATAGTGATTAA
120	ATATAACGTACATTGCAGCTGTAAATTGAATG
121	CGATTACTCCCATACCGGTGCTGGTGACGAGG
122	CGATCATGCTCGCGCCAGGCACGAGAACGTGG
123	CTCCTGCCTACGTCAGGCAATAGCAAACGAAT
124	AACTCCACTTCTACTCTTGTAGCGAGGCAACG
125	CATTTATCTTAATCACAGTAATCGCTGCGAAA
126	TACTTCCACCTCTCTTCGTTATATGCGATGTC
127	GCACTCGCCCTCGTCACATGATCGCGGAGACA
128	CAATATGTATTCGTTTAACATGGGCTCGGGTT
129	GTTCAGACCCACGTTCTTGCGGATACAAGAGC

Tables S4. Probe DNA sequences for making **Cuboid1**

DNA strand	Sequence (5'→3')
Probe1B-1	TTTTTTTTTTTCGCAGGTGGAGTTTTTTTTTTTTATCCTTA TCAAT
Probe1B-2	TTTTTTTTTGTCTCCGTGGAAGTATTTTTTTTTTTATCCTTA TCAAT
Probe1B-3	TTTTTTTTAACCCGAGGCGAGTGCTTTTTTTTTTTATCCTT ATCAAT
Probe1B-4	TTTTTTTTGACATCGCGCCAAGACTTTTTTTTTTTATCCTTA TCAAT
Probe1B-5	TTTTTTTTACCGTTGGGATAAATGTTTTTTTTTTTATCCTTA TCAAT
Probe1B-6	TTTTTTTTGCTCTTGTTGGACACATTTTTTTTTTTATCCTTA TCAAT
Probe1B-7	ATCCGCAACTGCCATCGACGAATGACGTGTGGCACGCG AATTTTTTTTTTATCCTTATCAAT
Probe1B-8	CCCATGTTCTTGCCCTAAGTCAAGAGACACAAGGCGTTT GTTTTTTTTTTATCCTTATCAAT
Probe1B-9	CCCTAGTTCCCAGATCTTGCAAGCTACGGGCGATCGGAA GTTTTTTTTTTATCCTTATCAAT
Probe1B-10	TTTTTTTTTCGTTCACGAGACATATTTTTTTTTTATCCTTA TCAAT
Probe1B-11	TTTTTTTTCGTTGCCTGAACTTGGTTTTTTTTTTATCCTTA TCAAT
Probe1B-12	TTTTTTTTTCCGTCACGTTTAAGGTTTTTTTTTTATCCTTA TCAAT
Probe1B-13	TTTTTTTTCACGCTACCGAGGTACTTTTTTTTTTATCCTTA TCAAT
Probe1B-14	TTTTTTTTGCAACCTGTGGGCGTGTTTTTTTTTTATCCTTA TCAAT
Probe1B-15	TTTTTTTTTACCGGATGCGCGGATTTTTTTTTTTATCCTTA TCAAT
Probe1B-16	TTTTTTTTACTTCGTAACATATTGTTTTTTTTTTATCCTTAT CAAT
Probe1B-17	TTTTTTTTTCAATACAGTCTGAACTTTTTTTTTTATCCTTA TCAAT
Probe1B-18	TTTTTTTTACCCGACGTGTGGTTATTTTTTTTTTATCCTTA TCAAT

Tables S5. Probe DNA sequences for making **Cuboid2**

DNA strand	Sequence (5'→3')
Probe2-1	TTTTTTTTTTTCGCAGGTGGAGTTTTTTTTTTTTTAACTATTC CTA
Probe2-2	TTTTTTTTTGTCTCCGTGGAAGTATTTTTTTTTTTTAACTATTC CTA
Probe2-3	TTTTTTTTAACCCGAGGCGAGTGCTTTTTTTTTTTTAACTATT CCTA
Probe2-4	TTTTTTTTGACATCGCGCCAAGACTTTTTTTTTTTTAACTATT CCTA
Probe2-5	TTTTTTTTACCGTTGGGATAAATGTTTTTTTTTTTTAACTATTC CTA
Probe2-6	TTTTTTTTGCTCTTGTTGGACACATTTTTTTTTTTTAACTATTC CTA
Probe2-7	ATCCGCAACTGCCATCGACGAATGACGTGTGGCACGCGAA TTTTTTTTTTTTAACTATTCCTA
Probe2-8	CCCATGTTCTTGCCCTAAGTCAAGAGACACAAGGCGTTTG TTTTTTTTTTTTAACTATTCCTA
Probe2-9	CCCTAGTTCCCAGATCTTGCAAGCTACGGGCGATCGGAAG TTTTTTTTTTTTAACTATTCCTA
Probe2-10	TTTTTTTTTCGTTCACGAGACATATTTTTTTTTTTTAACTATTC CTA
Probe2-11	TTTTTTTTCGTTGCCTGAACTTGGTTTTTTTTTTTTAACTATTC CTA
Probe2-12	TTTTTTTTTCCGTCACGTTTAAGGTTTTTTTTTTTTAACTATTC CTA
Probe2-13	TTTTTTTTCACGCTACCGAGGTACTTTTTTTTTTTTAACTATT CCTA
Probe2-14	TTTTTTTTGCAACCTGTGGGCGTGTTTTTTTTTTTTAACTATT CCTA
Probe2-15	TTTTTTTTTACCGGATGCGCGGATTTTTTTTTTTTTAACTATTC CTA
Probe2-16	TTTTTTTTACTTCGTAACATATTGTTTTTTTTTTTTAACTATTC CTA
Probe2-17	TTTTTTTTTCAATACAGTCTGAACTTTTTTTTTTTTAACTATTC CTA
Probe2-18	TTTTTTTTACCCGACGTGTGGTTATTTTTTTTTTTTAACTATTC CTA

3. Preparation of self-assembled DNA nanostructure

Design of DNA nanostructure. The 6H (helices)/6H/64BP (base pairs) nanostructure (**Cuboid1** and **Cuboid2**) was designed according to published methods developed previously,[S1] assisted by program Sequin[S2] (**Cuboid1**: DNA strands from Tables S3 and S4; **Cuboid2**: DNA strands from Tables S3 and S5). The 6H/6H/64BP nanostructure is a 6H by 6H by 64BP cuboid and 15 nm × 15 nm × 21.5 nm in size, with the addition of 8nt (nucleotides) repeat T at the head and tail of each helix to enhance its stability except for the **Probe1**- or **Probe2**-linking 18 helices (with a 10nt repeat T linker at the tail).

Preparation of DNA nanostructure. 147 DNA strands were mixed and freeze-dried. The dried DNA strand mixture was dissolved in 0.5 × TE buffer (5 mM Tris, 1 mM EDTA, pH 8.0) supplemented with 40 mM $MgCl_2$ to a final concentration of 200 nM per strand. The strand mixture was then annealed in a PCR thermocycler by a fast linear cooling step from 90 ^{o}C to 61 ^{o}C over 2.5 h, and subsequently from 60 ^{o}C to 24 ^{o}C over 74 h.

Purification of DNA nanostructure. Annealed samples were loaded onto 2% agarose gel in 0.5 × TBE buffer (44.6 mM Tris, 44.6 mM boric acid, 1 mM EDTA, pH 8.0) supplemented with 11 mM $MgCl_2$ and 0.5 μg/mL ethidium bromide. The gel was subjected to electrophoresis at 80 volts for 100 min in an ice water bath. Then, the target gel bands were visualized with ultraviolet light and excised. The excised bands were cut into small pieces and transferred into a Freeze 'N Squeeze column and the column was frozen at -20 ^{o}C for 5 min, then centrifuged at 7000 g for 5 min. Samples that were extracted through the column were collected and freeze-dried. The freeze-dried nanostructure was dissolved with water and the concentration of nanostructure was determined according to A_{260} prior to use.

Transmission electron microscopy (TEM) imaging. Unpurified or purified samples (2.5 μL) were loaded on a glass slide, and a carbon-coated TEM grid was placed on top of the samples for 2 min. The grid was then stained with 2% aqueous uranyl formate solution containing 25 mM NaOH for 2 min. The stained grid was washed

with water twice. Imaging was performed using a JEOL JEM-1011 operated at 100 kV.

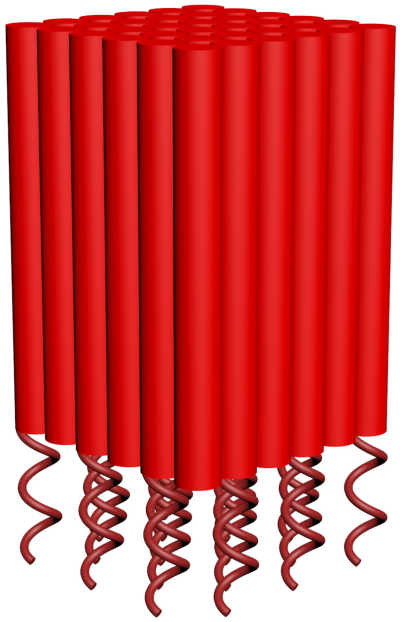

Fig. S1 3D model of the nanostructure 6H/6H/64BP.

Fig. S2 Locations of the 18 helices containing **Probe1** or **Probe2**. The 18 helices have been marked with white circles.

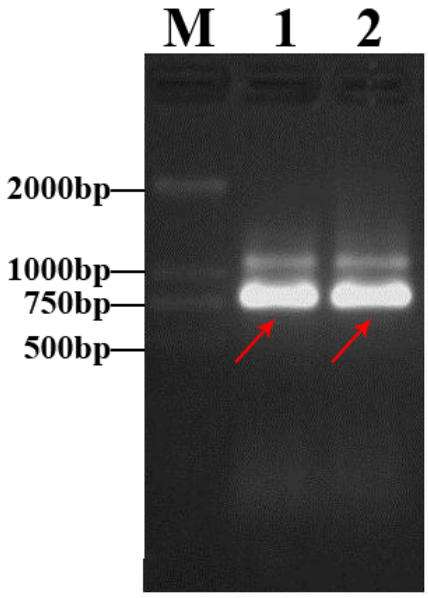

Fig. S3 Agarose gel electrophoresis diagram of the annealed **Cuboid1** and **Cuboid2**. Lane M: DL2000 DNA marker; lane 1: annealed **Cuboid1**; lane 2: annealed **Cuboid2**. The bands marked in red represent self-assembled DNA nanostructures.

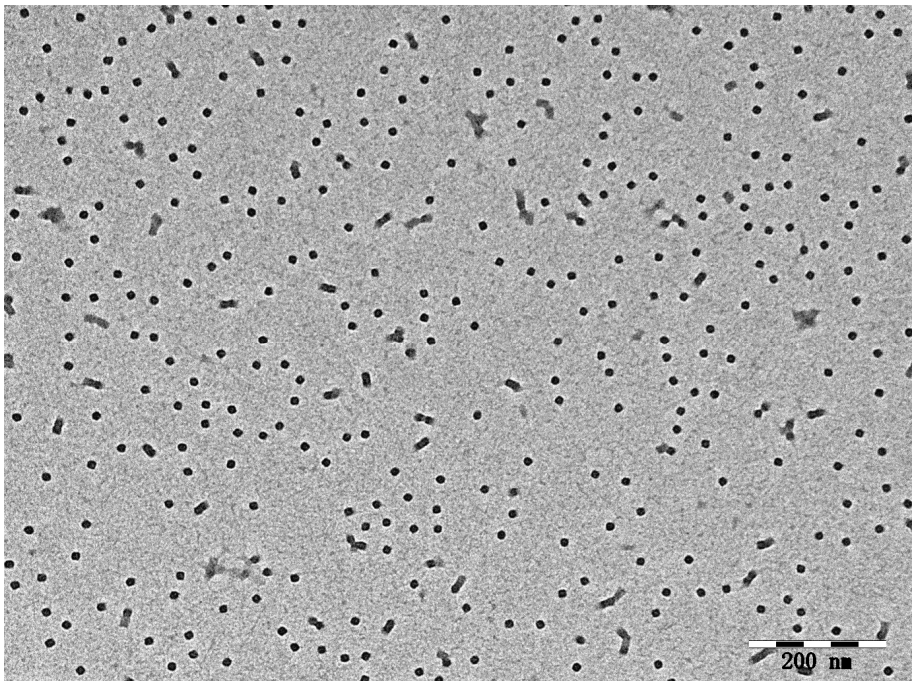

Fig. S4 TEM image of the annealed **Cuboid1**.

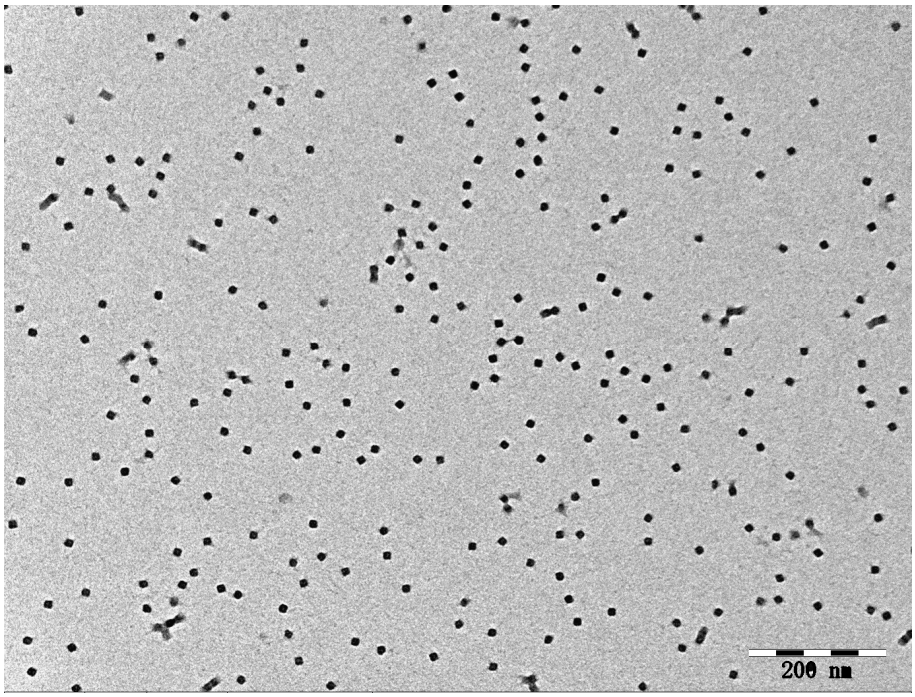

Fig. S5 TEM image of the purified **Cuboid1** as extracted from the band marked in the lane 1 of Fig. S3.

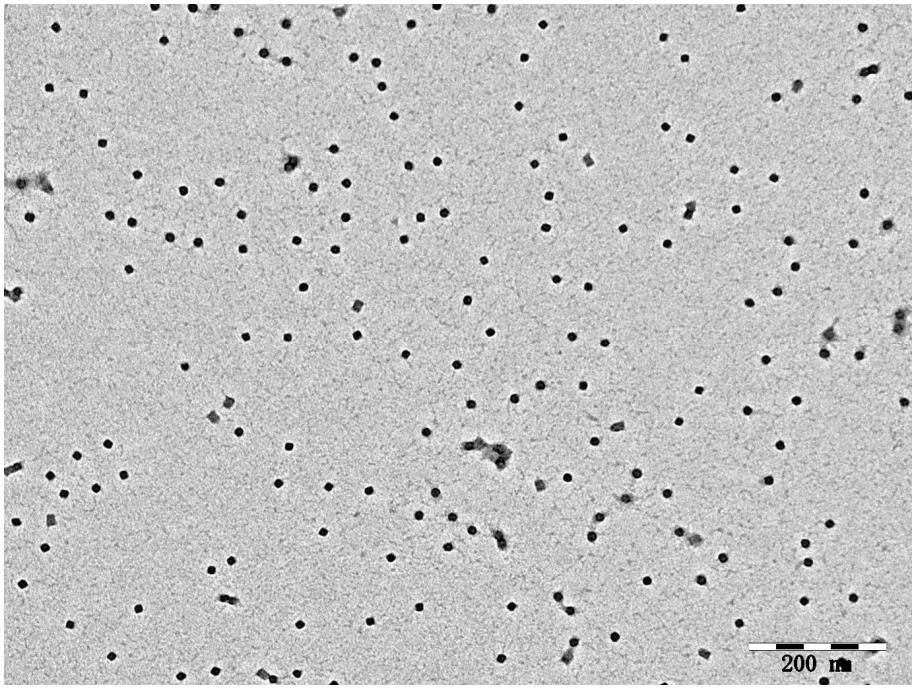

Fig. S6 TEM image of the annealed **Cuboid2**.

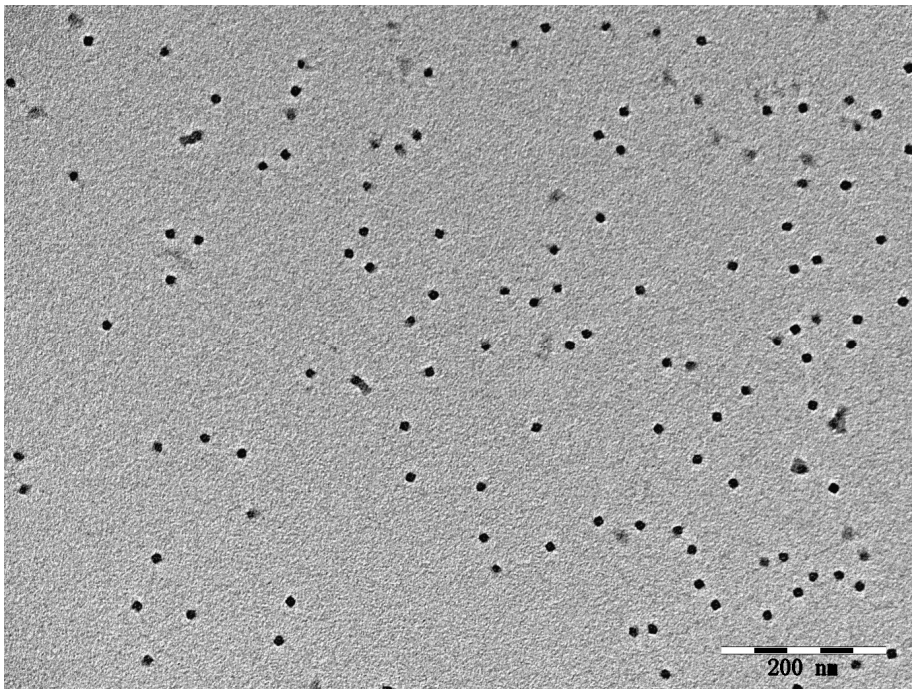

Fig. S7 TEM image of the purified **Cuboid2** as extracted from the band marked in the lane 2 of Fig. S3.

4. Stability of Cuboid1

Annealed **Cuboid1** (6 μL) was separately challenged with 54 μL of buffer solution A, B, and C. Buffer solution A: 0.5 × TE buffer supplemented with 40 mM $MgCl_2$. Buffer solution B: equal portions of 0.3 M PBS solution (0.3 M NaCl, 10 mM phosphate buffer, pH 7.0) and 5 × TBE buffer supplemented with 110 mM $MgCl_2$. Buffer solution C: 5 × TBE buffer supplemented with 55 mM $MgCl_2$. The diluted **Cuboid1** (60 μL, in buffer A, B, and C) was divided into two equal portions, each of which (30 μL) was placed at 4 ^{o}C and 37 ^{o}C for 12 h, and then 6 μL of 6 × loading buffer was placed in each solution. The mixture was loaded onto 2% native agarose gel in 0.5 × TBE buffer supplemented with 11 mM $MgCl_2$ and 5 μg/mL ethidium bromide. The gel was subjected to electrophoresis at 80 volts for 60 min in an ice water bath.

Annealed **Cuboid1** (3 μL) was separately challenged with 27 μL of buffer solution B, C, and D. Buffer solution D: equal portions of 0.5 × TE buffer supplemented with 40 mM $MgCl_2$ and 5 × TBE buffer supplemented with 110 mM $MgCl_2$. The diluted **Cuboid1** (30 μL, in buffer B, C, and D) was placed at 25 ^{o}C for 12 h, and then 6 μL of 6 × loading buffer was placed in each solution. The mixture was loaded onto 2% native agarose gel in 0.5 × TBE buffer (pH 8.0) supplemented with 11 mM $MgCl_2$ and 5 μg/mL ethidium bromide. The gel was subjected to electrophoresis at 80 volts for 60 min in an ice water bath.

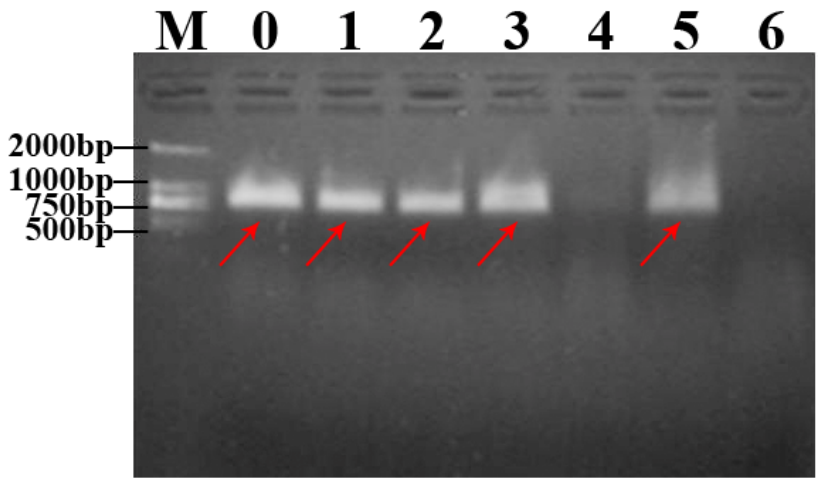

Fig. S8 Stability of **Cuboid1** in buffer solution A, B, and C at 4 ^{o}C and 37 ^{o}C. Agarose gel electrophoresis diagram showing stability of **Cuboid1** in buffer solution A, B, and C at 4 ^{o}C and 37 ^{o}C after 12 h. Lane M: DL2000 DNA marker; lane 0: annealed **Cuboid1**; lane 1: annealed **Cuboid1** in buffer solution A at 4 ^{o}C; lane 2: annealed **Cuboid1** in buffer solution A at 37 ^{o}C; lane 3: annealed **Cuboid1** in buffer solution B at 4 ^{o}C; lane 4: annealed **Cuboid1** in buffer solution B at 37 ^{o}C; lane 5: annealed **Cuboid1** in buffer solution C at 4 ^{o}C; lane 6: annealed **Cuboid1** in buffer solution C at 37 ^{o}C. The bands marked in red represent **Cuboid1.**

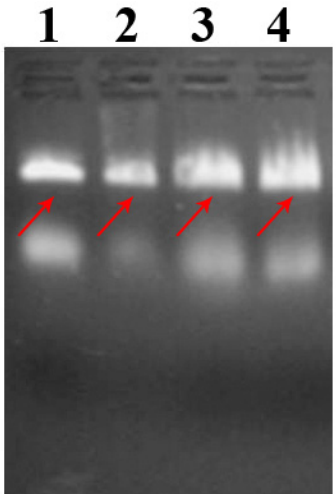

Fig. S9 Stability of **Cuboid1** in buffer solution B, C, and D at 25 ^{o}C. Agarose gel electrophoresis diagram showing stability of **Cuboid1** in buffer solution B, C and D at 25 ^{o}C after 12 h. Lane 1: annealed **Cuboid1**; lane 2: annealed **Cuboid1** in buffer solution B at 25 ^{o}C; lane 3: annealed **Cuboid1** in buffer solution C at 25 ^{o}C; lane 4: annealed **Cuboid1** in buffer solution D at 25 ^{o}C. The bands marked in red represent **Cuboid1.**

5. Preparation of DNA-modified glass slide

A glass slide was kept in a Piranha solution (hydrogen peroxide:concentrated sulfuric acid = 3:7, v/v) at 90 ^{o}C for 2 h. The glass slide was washed with copious amount of water and then placed in a mixture of hydrogen peroxide:ammonia solution:water (1:1:5, v/v/v) at 75 ^{o}C for 30 min. The glass slide was again washed with water and then placed in a mixture of hydrogen peroxide:hydrochloric acid:water (1:1:5, v/v/v) at 75 ^{o}C for 30 min. After this the glass slide was washed and incubated in an aqueous solution of EDAS (1% EDAS, v/v, 1 mM acetic acid) at room temperature for 30 min. After being washed with water, the EDAS-modified glass slide was N_2-dried and then baked at 120 ^{o}C for 30 min. After being cooled to room temperature, the glass slide was immersed overnight in 5 mM DMSO solution of SMCC at a temperature of approximately or slightly above 25 ^{o}C. The glass slide was washed with copious amount of ethanol and water, and then dried by N_2, and then immersed in a mixture of DMF:pyridine:hexanoic anhydride (4:1:1, v/v/v) at 4 ^{o}C for 2 h. The SMCC-modified glass slide was washed with ethanol and water, and then N_2-dried. Then 0.1 M PBS solution (0.1 M NaCl, 10 mM phosphate buffer, pH 7.0) of **Capture1** (10 μM, 3 μL for each spot) was spotted onto surface of the SMCC-modified glass slide and the reaction was allowed to proceed at 37 ^{o}C for 8 h in a humidity chamber. After being washed with water and dried with N_2, the glass slide was immersed in an ethanol solution of 1 mM 1-hexanethiol at room temperature for 12 h. Finally the glass slide was washed with ethanol and water, and then N_2-dried. The modification of **Capture2** and **RCA1** was performed in an analogous manner.

6. Detection of DNA1 with Cuboid1

A 0.3 M PBS solution (pH 7.0, containing 0.01 wt% SDS, 1.5 μL) of **DNA1** was spotted onto a **Capture1**-modified glass slide. Then a 5 × TBE buffer solution (1.5 μL) supplemented with 110 mM $MgCl_2$ of **Cuboid1** (100 nM) was added and the solution was thoroughly mixed. The hybridization process was performed at 25 ^{o}C for 12 h in a humidity chamber and then each spot on the glass slide was washed once with 50 μL of 1 M PBN (1 M $NaNO_3$, 10 mM phosphate buffer, pH 7.0) supplemented with 11 mM $MgCl_2$ and 0.05 wt% SDS, followed by another three times wash with 50 μL of pure 1 M PBN supplemented with 11 mM $MgCl_2$, and then the glass slide was placed in an oven at 40 ^{o}C for 4 min for the removal of water on the spots.

The image was recorded using a Canon DIGITAL IXUS 950IS digital camera. For image acquisition, the glass slide was intentionally suspended at an angle of 45 degrees in the air with the black photography backdrop placed away from the glass slide. The visible light source was positioned at the lateral upper side of the glass slide surface and the digital camera was placed in in parallel with the glass slide. The image acquisition was performed in a high-quality (3264 × 2448 pixels), lossy compression jpeg format. The utility of this format has enabled consistent, reproducible, and high-quality recording of macroscopic image under our experimental condition. The area of each salt spot on the glass slide in the image was calculated with the ImageJ software. The image was cut to the same size and the image type was changed from RGB color to 8-bit before the calculation.

7. Contact angle measurement

A 0.3 M PBS solution (pH 7.0, containing 0.01 wt% SDS, 4.5 μL) containing no DNA or **DNA1** (100 nM) was respectively spotted onto a **Capture1**-modified glass slide. Then a 5 × TBE buffer solution (4.5 μL) supplemented with 110 mM $MgCl_2$ of **Cuboid1** (100 nM) was added and the solution was thoroughly mixed. The hybridization process was performed at 25 ^oC for 12 h in a humidity chamber and then each spot on the glass slide was washed with 50 μL of 1 M PBN supplemented with 11 mM $MgCl_2$ and 0.05 wt% SDS, followed by another two times wash with 50 μL of pure 1 M PBN supplemented with 11 mM $MgCl_2$, and finally each spot was washed with 50 μL of water, and then N_2-dried. A 1 M PBN solution (3 μL) supplemented with 11 mM $MgCl_2$ was dropped onto the glass slide and contact angle was measured.

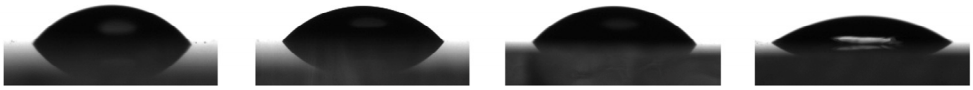

Fig. S10 Contact angle measured at different locations of the glass slide. Macroscopic image of salt solution droplet showing the contact angle at different locations of the glass slide. From left to right: **Capture1**-free area, **Capture1**-modified area, **Capture1**-modified area after hybridization in the absence of **DNA1**, **Capture1**-modified area after hybridization in the presence of **DNA1**.

Table S6. Contact angle at different locations of the glass slide. (ref. Fig. S10)

	Capture1-free area	**Capture1**-modified area	**Capture1**-modified area after hybridization in the absence of **DNA1**	**Capture1**-modified area after hybridization in the presence of **DNA1**
Contact angle (^{o})	53.4	49.0	49.8	34.2

8. Attempted detection of DNA1 with Probe1A or Probe1B mixture

A 0.3 M PBS solution (pH 7.0, containing 0.01 wt% SDS, 1.5 μL) of **DNA1** was spotted onto a **Capture1**-modified glass slide. Then a 5 × TBE buffer solution (1.5 μL) supplemented with 110 mM $MgCl_2$ of **Probe1A** (1.8 μM) or **Probe1B** mixture (100 nM per **Probe-1B** strand; for sequence information, see Table S4) was added and the solution was thoroughly mixed. The hybridization process was performed at 25 ^{o}C for 12 h in a humidity chamber and then each spot on the glass slide was washed once with 50 μL of 1 M PBN supplemented with 11 mM $MgCl_2$ and 0.05 wt% SDS, followed by another three or four times wash with 50 μL of pure 1 M PBN supplemented with 11 mM $MgCl_2$, and then the glass slide was placed in an oven at 40 ^{o}C for 4 min for the removal of water on the spots.

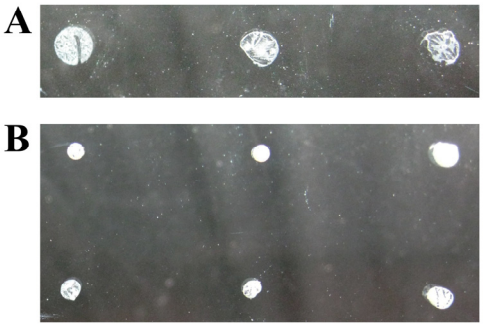

Fig. S11 Attempted DNA detection with **Probe1A**. A) Macroscopic image of a spot array on a glass slide for the attempted detection of **DNA1** with **Probe1A**. **DNA1** concentration (from left to right): 50 nM, 5 nM, and 0 M. The concentration of **Probe1A** is 900 nM. The glass slide was washed three times with 50 µL of pure 1 M PBN supplemented with 11 mM $MgCl_2$. B) Macroscopic image of a spot array on a glass slide for the detection of **DNA1** with **Probe1A**. **DNA1** concentration (from left to right, from top to bottom): 500 nM, 100 nM, 50 nM, 10 nM, 5 nM, and 0 M. The concentration of **Probe1A** is 900 nM. The glass slide was four times washed with 50 µL of pure 1 M PBN supplemented with 11 mM $MgCl_2$.

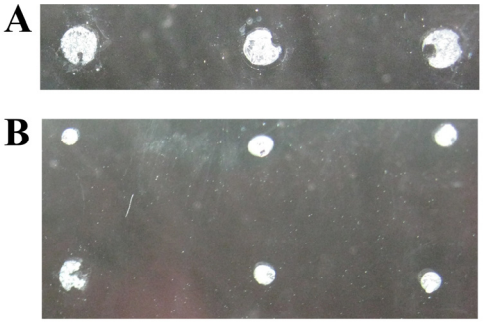

Fig. S12 Attempted DNA detection with **Probe1B** mixture. A) Macroscopic image of a spot array on a glass slide for the detection of **DNA1** with **Probe1B** mixture. **DNA1** concentration (from left to right): 5 nM, 0 M, and 0 M. The concentration of **Probe1B** mixture is 50 nM (50 nM per **Probe1B** strand). The glass slide was washed three times with 50 µL of pure 1 M PBN supplemented with 11 mM $MgCl_2$. B) Macroscopic image of a spot array on a glass slide for the detection of **DNA1** with **Probe1B** mixture. **DNA1** concentration (from left to right, from top to bottom): 100 nM, 50 nM, 25 nM, 10 nM, 5 nM, and 0 M. The concentration of **Probe1B** mixture is 50 nM (50 nM per **Probe1B** strand). The glass slide was washed four times with 50 µL of pure 1 M PBN supplemented with 11 mM $MgCl_2$.

9. Differentiation of DNA1 from DNA strands with single-base mismatches

A 75 mM PBS solution (75 mM NaCl, 2.5 mM phosphate buffer, containing 0.005 wt% SDS, pH 7.0, 3 μL) of either **DNA1** or a DNA strand with single-base mismatch (**DNA1-SBM1**, **DNA1-SBM2**, **DNA1-SBM3**, **DNA1-SBM4**) (50 nM) was spotted onto a **Capture1**-modified glass slide. The hybridization process was performed at 25 ^{o}C for 1 h in a humidity chamber and then each spot on the glass slide was washed with 50 μL of 60 mM PBS solution (60 mM NaCl, 2 mM phosphate buffer, pH 7.0) three times and then dried by N_2. A 0.5 × TBE buffer (1.5 μL) supplemented with 11 mM $MgCl_2$ of **Cuboid1** (20 nM) was spotted onto the dried spot and the hybridization process was performed at 25 ^{o}C for 1 h in the humidity chamber. Each spot was washed with 50 μL of 7.5 mM PBN (7.5 mM $NaNO_3$, 0.75 mM phosphate buffer, pH 7.0) supplemented with 1.25 mM $MgCl_2$ and 0.05 wt% SDS, followed by another three times wash with 50 μL of pure 7.5 mM PBN (pH 7.0) supplemented with 1.25 mM $MgCl_2$. Finally, each spot was washed with 50 μL of pure 1 M PBN supplemented with 11 mM $MgCl_2$, and then the glass slide was placed in an oven at 40 ^{o}C for 4 min for the removal of water on the spots.

10. Two-target detection

In a typical two-target detection experiment, a glass slide was spotted with 4 spots each of **Capture1** (top row) and **Capture2** (bottom row). The four columns of spots were each tested for a sample containing no DNA target, containing **DNA1** (100 nM), containing **DNA2** (100 nM), and containing both **DNA1** (100 nM) and **DNA2** (100 nM), respectively. The solutions of **Cuboid1** and **Cuboid2** were added to the 4 spots on the top and bottom rows, respectively. The hybridization was performed in an analogous manner (volume and concentration of each component except for DNA in the hybridization solution, duration of hybridization, concentration of each component in the wash buffer, and wash step) as that for the detection of single target.

11. RNA detection

RNA1 powder was dissolved with DEPC-treated 0.3 M PBS (0.01 wt% SDS, pH 7.0) and diluted to different concentrations. The freeze-dried **Cuboid1** was dissolved with DEPC-treated water and diluted with DEPC-treated 5 × TBE buffer solution (pH 8.0) supplemented with 110 mM $MgCl_2$ to 100 nM.

The **Capture1**-modified glass slide was washed with DEPC-treated water and dried by N_2. A DEPC-treated 0.3M PBS solution (pH 7.0, containing 0.01 wt% SDS, 1.5 μL) of **RNA1** was spotted onto the **Capture1**-modified glass slide. And then a DEPC-treated 5 × TBE buffer solution (pH 8.0, 1.5 μL) supplemented with 110 mM $MgCl_2$ of **Cuboid1** (100 nM) was added and the solution was thoroughly mixed. The hybridization process was performed at 25 ^oC for 4 h in a humidity chamber and then each spot on the glass slide was washed with 50 μL of 1 M PBN supplemented with 11 mM $MgCl_2$ and 0.05 wt% SDS, followed by another three times wash with 50 μL of pure 1 M PBN supplemented with 11 mM $MgCl_2$, and then the glass slide was placed in an oven at 40 ^oC for 4 min for the removal of water on the spots.

12. DNA detection through LCR-rolling circle amplification (LCR-RCA) protocol

In a typical DNA detection experiment, a 20 μL LCR solution containing **PDNA1** (1 μM), **AMPDNA1A** (1 μM), **AMPDNA1B** (1 μM), *Taq* DNA ligase (1 U/μL), *Taq* DNA ligase reaction buffer, and **DNA1** (various concentrations) was subjected to a thermal cycling treatment: 65 ^{o}C, 5 min/20 cycles of (65 ^{o}C, 30 sec/25 ^{o}C, 2 min/45 ^{o}C, 3 min)/65 ^{o}C, 7 min. Then the LCR mixture (20 μL) containing **CPDNA1** was subjected to enzymatic digestion in a final volume of 23.2 μL solution (exonuclease I 0.2 U/μL, exonuclease III 1.6 U/μL, 0.5 μL each of the two 10 × nuclease reaction buffer) at 37 ^{o}C for 1 h for the removal of single-strand DNA, **DNA1**, **PDNA1**, **AMPDNA1A**, **AMPDNA1B**, and ligation product from **AMPDNA1A** and **AMPDNA1B**. The exonucleases were heat inactivated by incubation at 80 ^{o}C for 15 min.

A RCA reaction solution was prepared through the mixing of following solutions: 10 mM dNTPs (2 μL), 10 × φ29 DNA polymerase reaction buffer (2 μL), and φ29 DNA polymerase (1 μL). The RCA reaction solution (0.65 μL) was added to LCR mixture (3 μL) containing **CPDNA1** and the solution was thoroughly mixed. The above mixture (3 μL) was spotted onto the **RCA1**-modified glass slide. The RCA reaction process was allowed to perform at 37 ^{o}C for 5 h in a humidity chamber and then each spot on the glass slide was washed with 50 μL of 1 M PBN supplemented with 11 mM $MgCl_2$ and 0.05 wt% SDS, followed by another six times wash with 50 μL of pure 1 M PBN supplemented with 11 mM $MgCl_2$, and then the glass slide was placed in an oven at 40 ^{o}C for 4 min for the removal of water on the spots.

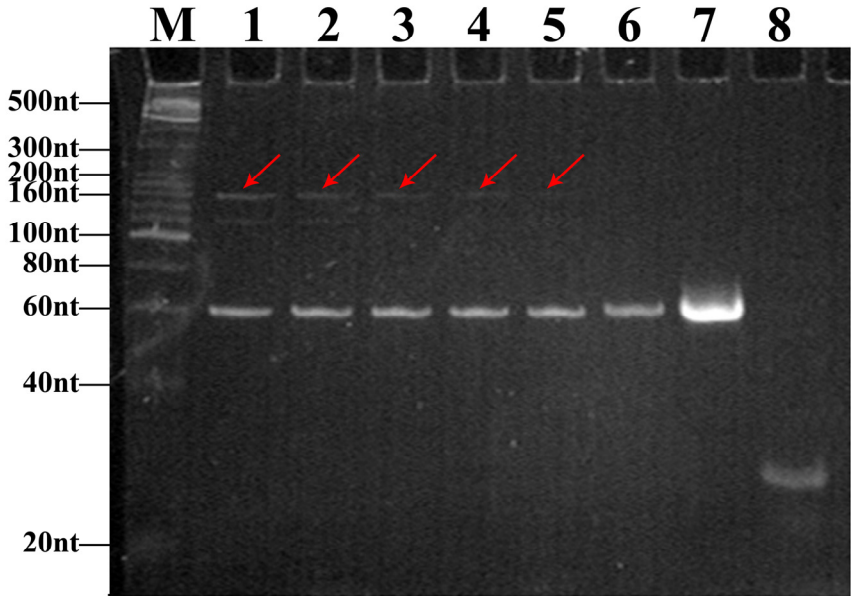

Fig. S13 Verification of the ligation reaction by *Taq* DNA ligase. Gel electrophoresis diagram showing the ligation of **PDNA1** by *Taq* DNA ligase in the presence of **DNA1**. Lane M: DNA ladder marker; lanes 1 to 6: **DNA1** concentration of 100 nM, 10 nM, 1 nM, 100 pM, 10 pM, 0 M; lane 7: **PDNA1**; lane 8: **DNA1**. The bands marked in red represent the ligation product **CPDNA1**. **PDNA1** (1 μM), **AMPDNA1A** (1 μM), and **AMPDNA1B** (1 μM) were ligated by *Taq* DNA ligase (1 U/μL) in the presence of **DNA1** (various concentrations) under LCR condition: 65 ^{o}C, 5 min/20 cycles of (65 ^{o}C, 30 sec/25 ^{o}C, 2 min/45 ^{o}C, 3 min)/65 ^{o}C, 7 min.

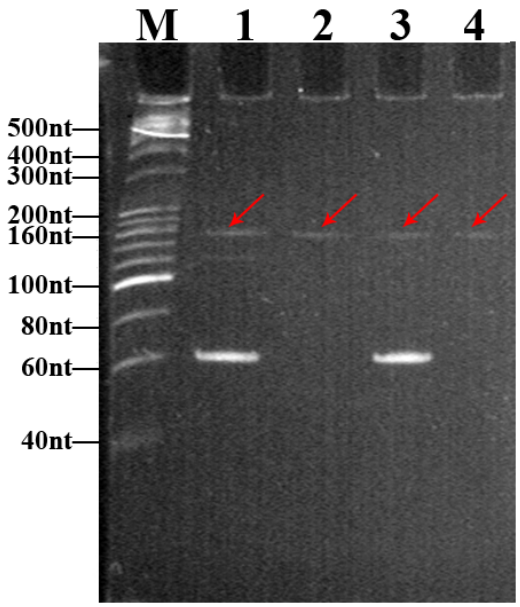

Fig. S14 Verification of enzyme digestion of exonuclease I and exonuclease III. Gel electrophoresis diagram showing the ligation of **PDNA1** and enzymatic digestion with exonuclease I and exonuclease III. Lane M: DNA ladder marker; lane 1: ligation mixture (concentration of **DNA1** is 100 nM**)** before enzymatic digestion; lane 2: ligation mixture (concentration of **DNA1** is 100 nM**)** after enzymatic digestion; lane 3: ligation mixture (concentration of **DNA1** is 10 nM**)** before enzymatic digestion; lane 4: ligation mixture (concentration of **DNA1** is 10 nM**)** after enzymatic digestion. The bands marked in red represent the ligation product **CPDNA1**.

13. References

S1. Y. Ke, L. L. Ong, W. M. Shih, P. Yin, *Science*, 2012, **338**, 1177-1183.

S2. N. C. Seeman, *J. Biomol. Struct. Dyn.*, 1990, **8**, 573-581.