Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Design and Construction of Reconfigurable DNA Nanocage to Encapsulate TMV Disk

Tianran Zhang[a], Xiangzhi Zeng[a], Shuwen Guan[b], Xiumei Li[a], Zhiyu Qu[a], Luyao Qin[a], Chunxi Hou[a] & Junqiu Liu[a]**

a. State Key laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, 2699 Qianjin Street, Changchun 130012, China, Fax:(+86)85168452
b. College of Life Science, Jilin University, 2699 Qianjin Street, Changchun 130012, China

*Email:junqiuliu@jlu.edu.cn
chunxihou@jlu.edu.cn

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1. Design of DNA nanocage

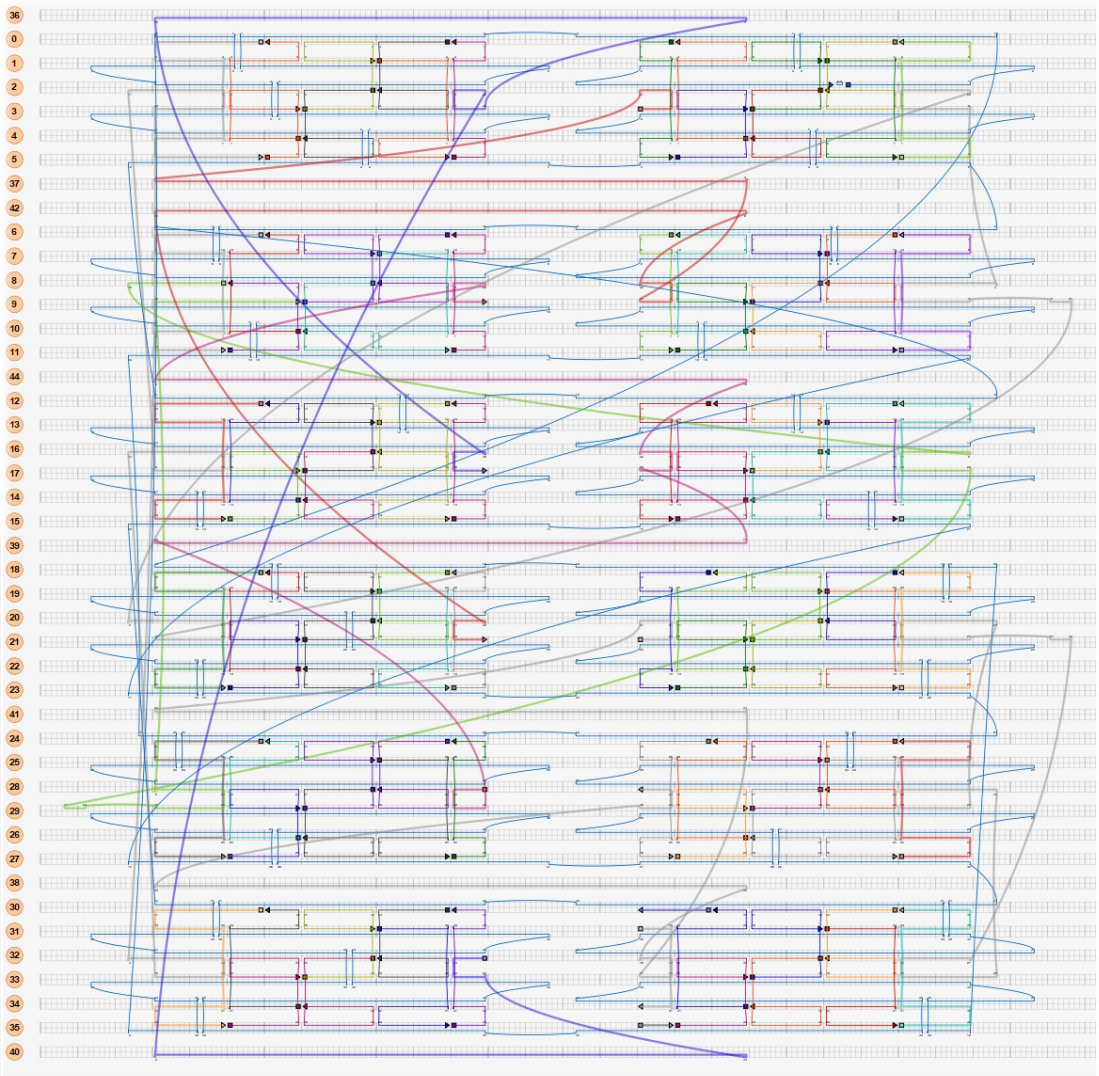

Figure S1 Design of DNA nanocage in caDNAno

Start	End	Sequence
32[146]	34[133]	CTTTGAAAGAGGACTAATAGTAAAATGTAATATTCATTGAAT
31[112]	35[118]	GGCTGACTTAAACAAAGAAGT
15[161]	12[161]	AAAAAAGGCTCCAATTTTTTCACGTTGACAAAGACAAAAGG GAATAAGTTTATTTT
20[146]	22[133]	AAACTCCAACAGGTTCATATATTTTAAAGGTGAGAAAGGCCG
35[161]	30[161]	GTCCAGAAGCCCGAGATTAAGAGATACTCCAAATCAAGAAC GAGCCGCGTAACAAA
27[119]	29[132]	GACTTTTTCATGAGATTTAAATTGTAAAACCTAAAACGAAAG
27[77]	24[77]	CGGCTACAGGGAGTAACCGTGTCATCAA
8[62]	10[49]	CCTTATTACGCAGTGACTCTAGAGGATCCGGCCAGTGCCAAG
30[76]	32[63]	GATGGTTTAATTTCAAAGATTCATCAGTACGGAACAACATTA
28[167]	20[168]	AAAGCCCCTTTGACCCCCAGCTTTTTGAGCTTC
24[41]	27[34]	TCCGTGGGAACAAACGAGGCAAAACCGCGGATTGATCGAGG TGAATTTCGCCTTTAATTGTAT
6[41]	11[34]	AAAGTAAGCACGCAAAGACACCATATAAAAGAAAGTTGGGA AGGGCGATTTCGCCATTCAGGC
3[133]	1[146]	GAGGTTTTGAAGCCCCAGTCATGTAATTCAACGCCAATTGGA
23[77]	18[77]	AGCCTTTCAAGGCATATTTTCCAATTCT
31[63]	35[76]	GGACGTTGGGAAGATAATGCAGATACATGATAAAAACCAAAA
18[76]	20[63]	GCGAACGAGTAGATGTAGTAGCATTAACAAGGTGGCATCAAT
31[147]	35[160]	CGGATATTCATTACGCGGAATCGTCATATTAGACTGGATAGC
20[83]	9[118]	AGCTGAAATCCAATGGAGACATAACGCGTAACAATCGCGAAA ACGCGTAACAATCGCGAAAACGCGTAACAATCGCGAAAACGC GTAACAATCGCGAAAACGCGTAACAATCGCGAAGACATGCGC CAATTCC
23[119]	21[132]	AATTTTTAGAACCCCAGGATTAGAGAGTTAGCTGATAAATTA
12[41]	15[34]	CCGCCACCCTCAGAGCCACCACTTTTCATAATCAAATTTTGCT CAGTACCGGGGTTTTCAGGG
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13[63]	15[76]	ACCGCCACCGTAATACCGCCACCCTCAGCGTAACACTGAGTT
21[133]	19[146]	ATGCCGGAGAGGGTCTAAAGTACGGTGTATGGCTTAGAGCTT
4[132]	0[119]	CTTATCCGGTATTCATCGCCATATTTAATAGGCAGAGGCATT
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32[167]	28[168]	TAAGGGAAGAAGCAAAGCGGATTTTTAATCAGA
2[27]	16[28]	TGAATTTTTTTTGCCGTCGAGAGGGATAGCCC
30[125]	30[112]	GAAACACCAGAACG

10[48]	6[42]	CTTGCATGCAACTGATAGCCGAACAAAGTTTAAGA
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8[83]	9[83]	AGAACTGGTAACGC
13[147]	15[160]	TGGTTTACCAGCGCAAATCCGATCTAAATTAGCGTAATCCAA
34[48]	30[42]	TTACGAGGCATAGTTTTAAGAACTGGCTTGAATTA
18[125]	23[118]	TTTCATTCCATATAGATAAGAACCATCAGGATAAA
9[49]	7[62]	GGGATGTGCTGCAATTACCGAAGCCCTTTTACCAGAAGGAAA
27[35]	29[48]	CGGTTTATCCAGCGCGCACTCCAGCCAGGCCGACAATGACAA
1[147]	5[160]	ACAAGAGTCCACTATACCAACGCTAACGTCTTTCCAGAGCCT
2[62]	4[49]	CCATCCTAATTTACCCGCCGCCAGCATCCATTGACAGGAGGT
0[118]	5[118]	TTCGAGCATTGAGATAAGAACGGAATCA
8[118]	2[112]	AGAATTATCAGACATAACCCCTAACAATGGGGAAAACCCCTA ACAATGGGGAAAACCCCTAACAATGGGGAAAACCCCTAACA ATGGGGAAAACCCCTAACAATGGGGAAGACATGGCTAGCGA ACCGCTCAA
5[119]	3[132]	TTACCGCGCCCAATCAGTATAAAGCCAACTCCCGACTTGCGG
21[49]	19[62]	TGGCCCTGATAATATTAGTTTGACCATTCGCAAATGGTCAAT
20[167]	32[168]	AAAGCGAGATCTACAAAGGCTTTTTTTCAATCA
6[76]	8[63]	TAGCAATAGCTATCGGCGATTAAGTTGGGCATGATTAAGACT
33[112]	29[118]	AGAATGACAGACCAGGAGACATAACGCGTAACAATCGCGAA AACGCGTAACAATCGCGAAAACGCGTAACAATCGCGAAAAC GCGTAACAATCGCGAAAACGCGTAACAATCGCGAAGACATG CGCGGCACCA
12[76]	16[63]	CGATAGCAGCTCCCTATCACCGTACTCATTAGCGTCAGACTG
28[118]	20[112]	CGTTAATTCAGACATAACCCCTAACAATGGGGAAAACCCCTA ACAATGGGGAAAACCCCTAACAATGGGGAAAACCCCTAACA ATGGGGAAAACCCCTAACAATGGGGAAGACATGGCTACCGT TCACCTTTA
19[147]	23[160]	AATTGCTGAATATATAAAGATTCAAAAGTGCAATGCCTGAGT
12[125]	15[118]	TAGCAAGGCCGGAAATTTGGGATGAATTCAACGCC
23[161]	18[161]	AATGTCTGGAGCAACCTGAGAGTGTAGGATGCTGTAGATATC TTCAACTCAACATG
24[160]	28[147]	GTAATCGTAAATAGACTAAAACACTCATCAAAAACAGGAAGA
10[132]	6[119]	TGTCGTGCCCTGTGGTCAGAGGGTAATTATTGAGTTAAGCCC
17[112]	16[112]	TTTTGCTACCGTCA
16[146]	14[133]	GGAAATTATTCATTGACAGCCCTCATAGGTTTTGTCGTCTTT
1[63]	5[76]	AGAACGCGCCTGTTCCAAGTACCGCACTGAGAACAAGCAAGC
35[119]	33[132]	TTTGCCAGAGGGGGAGATGAACGGTGTACCATAAATCAAAAA
29[133]	25[146]	AGGCAAAAGAATACGAACGCCATCAAAAATCAGCTCATTTTT
26[132]	24[126]	TTAAACGGGTAAAATAAATTTTTGTTAAATAATTC
32[27]	2[28]	TGAAAGTTTTTTATAAATCCTCATTACAGTCTC
4[48]	0[42]	TGAGGCAGGTCAGACTCAATAAACAACATCCAGAC
28[83]	29[83]	TGAGGGGTTCGGTC
11[35]	8[35]	TGCGCCTGCAGGTCATGTTAGCAAACGT

17[49]	13[62]	GCCCGGAATAGGTGTCAGAGCCGCCACCGAGCCACCACCGGA
12[160]	16[147]	GTCACAATCAATAGGAGAATAGAAAGGAAGGTAAATATTGAC
29[49]	25[62]	CAACCATCGCCCACGAGTAACAACCCGTGTCACGTTGGTGTA
28[146]	26[133]	TTGTATAAGCAAATGAAGTTGATAAATTATCATCGCCTTCCA
15[77]	12[77]	TCGTCACCCTCAGACAGTAGCAAACCAT
16[62]	14[49]	TAGCGCGTTTTCATAGGAACCCATGTACAACCGCCACCCTCA
16[167]	28[28]	GGAGGGAACAACTAAAGGAATTTTTTATACCGATAGTTGCCTT TCCG
0[76]	2[63]	AGTACCGACAAAAGTCGGCTGTCTTTCCAGAAAAATAATATC
2[83]	16[77]	CTGAACATTATCATGGAGACATAACGCGTAACAATCGCGAAA ACGCGTAACAATCGCGAAAACGCGTAACAATCGCGAAAACG CGTAACAATCGCGAAAACGCGTAACAATCGCGAAGACATGC GCTTTGCCT
25[63]	27[76]	GATGGGCGCATCGTTAAAGGCCGCTTTTAACGAGGGTAGCAA
33[133]	31[146]	TCAGGTCTTTACCCGAATAAGGCTTGCCATCTTGACAAGAAC
34[132]	30[126]	CCCCCTCAAATGCTCTTCATCAAGAGTACTGACGA
3[49]	1[62]	AAACCAATCAATAAGTAAAGTAATTCTGTGTTCAGCTAATGC
16[27]	32[28]	CCTTATTTTTTTTGAGTAACAGTGCCTGAAACA
25[147]	27[160]	TAACCAAACTAGCACAACGGAGATTTGTGTGTCGAAATCCGC
26[48]	24[42]	CTCAGAGCTTGCTTCCGTAATGGGATAGCGGATTC
18[160]	20[147]	TTTTAAATATGCAAAGCTATTTTTGAGAACCAGACCGGAAGC
20[27]	2[168]	TGCCCCATTTTTAGGGCGA
0[160]	2[147]	GGTTGAGTGTTGTTTTAAATCAAGATTATCTATCAATGCGTT
15[119]	17[132]	TGTAGCATTCCACAAAAGGTGAATTATCAAACAACTTTCAAC
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8[167]	20[28]	AGCCTTTTGCCTAATGAGTGATTTTTGGGCAACAGCTGATGC TGGTT
28[27]	9[48]	GCACCGCTTTTTCTTCGCTATTACGCCAGCTGGCGAAAGG
15[35]	17[48]	ATAGCAAGCCCAATCGGCATTTTCGGTCTTGATATAAGTATA
32[62]	34[49]	TTACAGCCTATTTCGTTTACCAGACGACAACGCCAAAAGGAA
14[48]	12[42]	GAGCCACCACCCTCAATCACCGGAACCACTCAGAA
8[146]	10[133]	ATAAAAACAGGGAAAATGAATCGGCCAACCAGTCGGGAAACC
17[77]	33[83]	GGAGGTTTCAGACATAACCCCTAACAATGGGGAAAACCCCTAA CAATGGGGAAAACCCCTAACAATGGGGAAAACCCCTAACAAT G GGGAAAACCCCTAACAATGGGGAAGACATGGCTCGAACTATG A GATT
0[41]	5[41]	GACGATTTGATGATACAGGAGAGCGTCATACATGGCGATTGGC C TTGATAGAGCCGCCACCAGAACCACC
30[160]	32[147]	GCTGCTCATTCAGTTGACTATTATAGTCACCGAACTGACCAA
24[76]	28[63]	CATTAAATGTGAGCGCATAACCGATATAACGACGACAGTATC
8[34]	16[168]	AGAAAATACATACATTTTTGATTGAG

5[42]	3[48]	ACCAGAGGAGCATGGAAAGCGAAGCCAGAATGTAG
2[146]	4[133]	ATACAAATTCTTACAGCAAGCAAATCCGAGAGATATAGAAGG
5[161]	0[161]	AATTTGCCAGTTACTTTATCCTGAATCTTTAAAGAACGTGGAA TAGCCCGAGATAG
17[133]	13[146]	AGTTTCAGCGGAGTAAAATGTAGCACCAAAATCACCATCATA
7[63]	11[76]	CCGAGGAAACGCAAACGTTGTAAAACGACCCGGGTACCGAGC
20[62]	22[49]	TCTACGAGAGTTGCTATGACCCTGTAATTTAAGCAATAAAGC
11[77]	6[77]	TCGAATTAGTCACGTAATAACAATGAAA
18[41]	23[34]	GGCAAAATCCCTTATAAATCACCTGTTTGATGGTGCTAAATTTT CTTTTCAGGGTGGTTCGGT
5[77]	0[77]	CGTTTTTTATTAAATATCAACAATATAA
30[41]	35[34]	CCTTATGCGAGAAGGATTAGGAGACTCCTCAAGATAAGAGGTT TTAACGTGGTAATAACAACA
24[125]	27[118]	GCGTCTGGCCTTCCTTCGCATTACGTAAGACTAAA
6[118]	11[118]	AATAATAGAACAAATGAAATTCATAGCT
35[77]	30[77]	TAGCGAGATTCAACAAAATCTGGCTTGA
22[132]	18[126]	GAGACAGTCAAATCGGTCATTTTTGCGGCTGGAAG
33[49]	31[62]	ATGCCCCCTGGTAGAACTTTAATCATTGCATTATACCAGTCA
19[63]	23[76]	AACCTGTTTAGCTAAAGAATTAGCAAAAACTTTTGCGGGAGA
23[35]	21[48]	TGTACCAAAAACATAGCAAGCGGTCCACTGCCCTTCACCGCC
22[48]	18[42]	CTCAGAGCATAAAGGTTCCGAAACATTTAGATATC
28[62]	26[49]	GGCCTCAGGAAGATAAAGACAGCATCGGGCGGGATCGTCACC

Table S1 Sequence of staple strands

start	end	Hairpin sequence
20[83]	9[118]	AGCTGAAATCCAATGGAGACATAACGCGTAACAATCGCGAAAACG CGTAACAATCGCGAAAACGCGTAACAATCGCGAAAACGCGTAACA ATCGCGAAAACGCGTAACAATCGCGAAGACATGCGCCAATTCC
8[118]	2[112]	AGAATTATCAGACATAACCCCTAACAATGGGGAAAACCCCTAACA ATGGGGAAAACCCCTAACAATGGGGAAAACCCCTAACAATGGGGA AAACCCCTAACAATGGGGAAGACATGGCTAGCGAACCGCTCAA
33[112]	29[118]	AGAATGACAGACCAGGAGACATAACGCGTAACAATCGCGAAAAC GCGTAACAATCGCGAAAACGCGTAACAATCGCGAAAACGCGTAAC AATCGCGAAAACGCGTAACAATCGCGAAGACATGCGCGGCACCA
28[118]	20[112]	CGTTAATTCAGACATAACCCCTAACAATGGGGAAAACCCCTAACA ATGGGGAAAACCCCTAACAATGGGGAAAACCCCTAACAATGGGGA AAACCCCTAACAATGGGGAAGACATGGCTACCGTTCACCTTTA
2[83]	16[77]	CTGAACATTATCATGGAGACATAACGCGTAACAATCGCGAAAACG CGTAACAATCGCGAAAACGCGTAACAATCGCGAAAACGCGTAACA ATCGCGAAAACGCGTAACAATCGCGAAGACATGCGCTTTGCCT
17[77]	33[83]	GGAGGTTTCAGACATAACCCCTAACAATGGGGAAAACCCCTAACA ATGGGGAAAACCCCTAACAATGGGGAAAACCCCTAACAATGGGGA AAACCCCTAACAATGGGGAAGACATGGCTCGAACTATGAGATT

Table 2 Sequence of hairpins

name	sequence
Fuel Strand 1	CCCGCGATTGTTACGCGCC
Antifuel Strand 1	GGCGCGTAACAATCGCGGGGGCGCGTAACAATCGCGGGGGCGCGTA ACAATCGCGGGGGCGCGTAACAATCGCGGGGGCGCGTAACAATCGC GGG
Fuel Strand 2	CCCCCCATTGTTAGGGGCC
Antifuel Strand 2	GGCCCCTAACAATGGGGGGGGCCCCTAACAATGGGGGGGGCCCCTA A CAATGGGGGGGGCCCCTAACAATGGGGGGGGCCCCTAACAATGGGG G G

Table S3 Sequence of fuel strands and antifuel strands

The design of whole DNA nanocage is finished using caDNAno (Figure S1) with honeycomb-pleated pattern. 112 short DNA staples with detail sequence will be autogenerated from caDNAno (Table S1). Two different sequences of hairpin and the corresponding fuel strands and antifuel strands are listed in Table S2 and Table S3 respectively. The hairpins are designed under the theory of persistence length that is a basic mechanical property quantifying the stiffness of a polymer. Generally, a piece of polymer with physical length shorter than its persistence length will behave like a rigid rod, while for a piece of polymer with physical length longer than its persistence length will behave like a flexible rope[1]. Since the persistence length of double stranded DNA ranges from 100bps to 160bps[2] and the length of hairpin in DNA nanocage is 133bps, the opening hairpins are theoretically rigid enough to maintain the open state of DNA nanocage. The extreme flexibility of thymine-rich DNA single strand at joints can reduce the hinder generated during the transformation of DNA nanocage. Thus, the addition of anti-fuel DNA will remove the fuel and leave the hairpins structure forming again to drive the rods close together, which results in the closed state.

2. Synthesis of DNA nanocage and TMV disk-nanocage complex

2.1 Materials

All staple strands, primers, anti-primers and M13mp18 viral DNA for the folding of the DNA nanocage were purchased and commercially synthesized from Changchun Huada Zhongtian Biotechnology Co., Ltd, and were used without further purification.

2.2 Generation and characterization of scaffold

Asymmetric PCR (α PCR) was performed to minimize the excess ssDNA in the final structure. 1 mM sense primer, 20 nM anti-sense primer, 50 ng M13mp18 dsDNA template, 200 mM dNTPs were mixed in a final volume of 50 μl and 1 unit of Q5TM High-Fidelity DNA Polymerase (NEB) was then added. The aPCR program is set as follows: 95℃, 4 min for the initial denaturation; followed by 35 to 40 cycles of 95℃, 30 s; 55℃, 30 s; 72℃, 5min; 72℃for final extension, and hold at 4℃.

Name	Sequence (5'-3')
Sense	CGCATAGAATTCTAAACAGGCT
Anti-sense	CGAAAAACCGTCTATCAAGATCTCT

Table S4 Sequence of sense and anti-sense

2.3 Protocol of DNA nanocage synthesis

Staples, scaffold, TAE and Mg^{2+}-containing buffer were mixed together as the Table S4 shows. Then the mixture was heated up to 80℃ at first, then slowly cooled down from 80℃ to 60℃ at 4min/℃, and then 60℃ to 24℃ at 20min/℃. The obtained DNA nanocage was stored at 4℃.

Name	Concentration	[μl]	Final concentration
Scaffold	10~20 nM	25	5~10 nM
Staple	167~333 nM	15	50~100 nM
10X TAE	——	5	1× TAE
10× Mg2+ buffer	100~200 mM	5	10~20 mM

Table S5 concentration and volume of materials for DNA nanocage synthesis

2.4 Conformational alternation

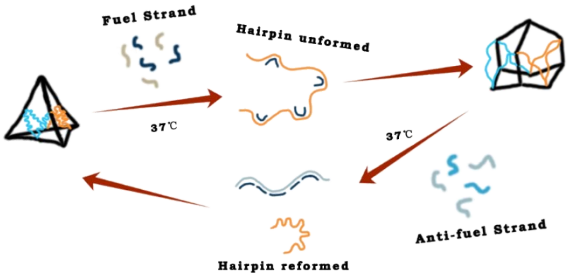

Figure S2 Protocol of DNA nanocage conformational alternation

The conformational alternation of DNA nanocage is carried out as Figure S2 shows: To change DNA nanocage from closed state to open state, 25μl DNA nanocage in closed state solution was mixed with 25μl fuel strands solution. The concentration of fuel strands is 15 times of DNA nanocage. The mixture was then incubated at 30℃ for 30min and cool down to 15℃ at 3℃/min. To change DNA nanocage from open state to closed state, all the protocol was identical with the former, except the closed state being replaced by open state and fuel strands being replaced by antifuel strands.

2.5 Capture of TMV disk

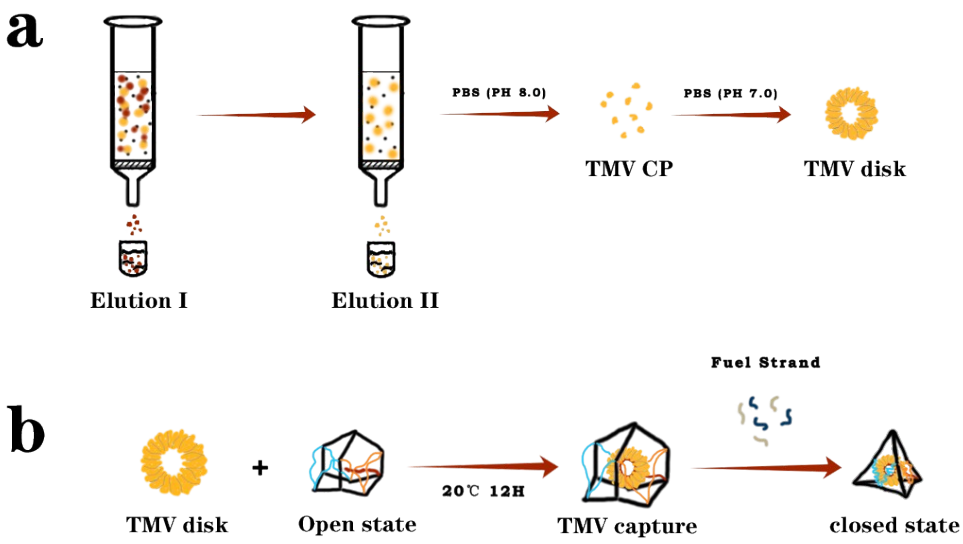

Figure S3 Protocol of (a) TMV disk purification and assembly; (b) capture of TMV disk by DNA nanocage.

The TMV CPs purified by DEAE anion exchange chromatography were disaggregated through dialyzing in the PBS buffer (PH 8.0) at 4℃ for 48h, the disaggregated proteins were then dialyzed in PBS buffer (PH 7.0) at 4℃ for more than 24h. The assembled TMV disks were incubated with the DNA nanocage in open state at 20℃, 12h. Then the mixture was added with antifuel strands and incubated for another 40 min to close the nanocage.

3. Characterization methods of DNA nanocage and TMV disk-nanocage complex

3.1 Electrophoresis imaging

The samples were run through 0.8% low-melting temperature agarose gel (0.2g agarose, 25ml 1× TAE) under a constant voltage of 80V. The products were then extracted and purified by Gel DNA recovery kit.

3.2 AFM imaging

The DNA nanocage sample (5 μL) was deposited onto a freshly cleaved mica and left to adsorb for 5 min. The sample was washed with ddH2O to remove the excess salt, and then air dried. The sample was scanned in a tapping mode on a Bruker AFM.

3.3 TEM imaging

The TEM sample was prepared by dropping 5μl of the sample solution on carboncoated grid for 5min. The grid was touched with a drop of 2 % uranyl acetate solution and excess solution was wicked away with a filter paper. To evaporate extra solution, the grid was kept at room temperature. TEM studies were conducted by using a JEM-2100F transmission electron microscope, operated at 200 kV in the bright field mode.

3.4 DLS imaging

Measurement were made on ALV/CGS-3 compact goniometer made by ALV. 100μl sample solutions were measured at a concentration of 10nM in 1× TAE Buffer. The sample temperature was maintained at 25℃ during measurement.

4. characterizations of DNA nanocage and TMV disk-nanocage complex

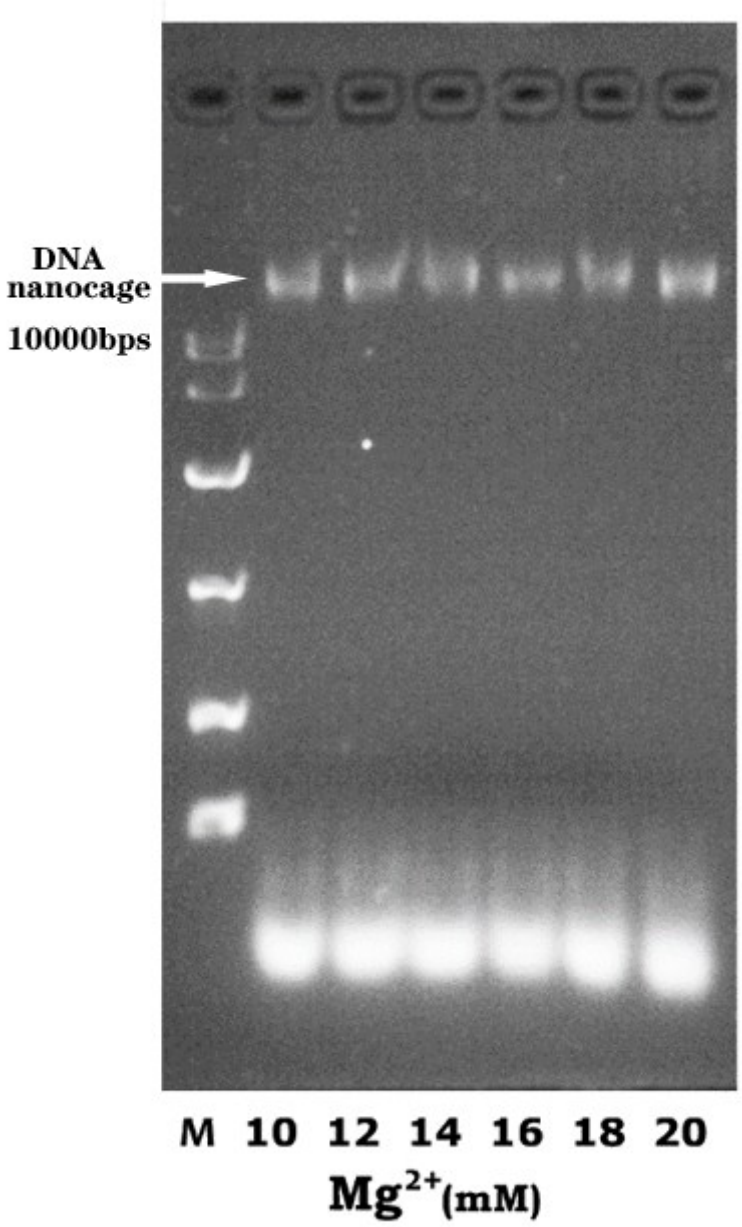

Figure S4 Gel analysis of DNA nanocage assembled in gradient Mg^{2+} concentration.

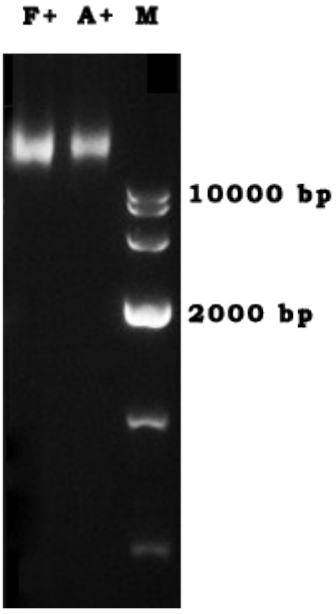

Figure S5 Gel analysis of DNA nanocage in open state after the addition of fuel strands (F+) and closed state after the addition of antifuel strands (A+).

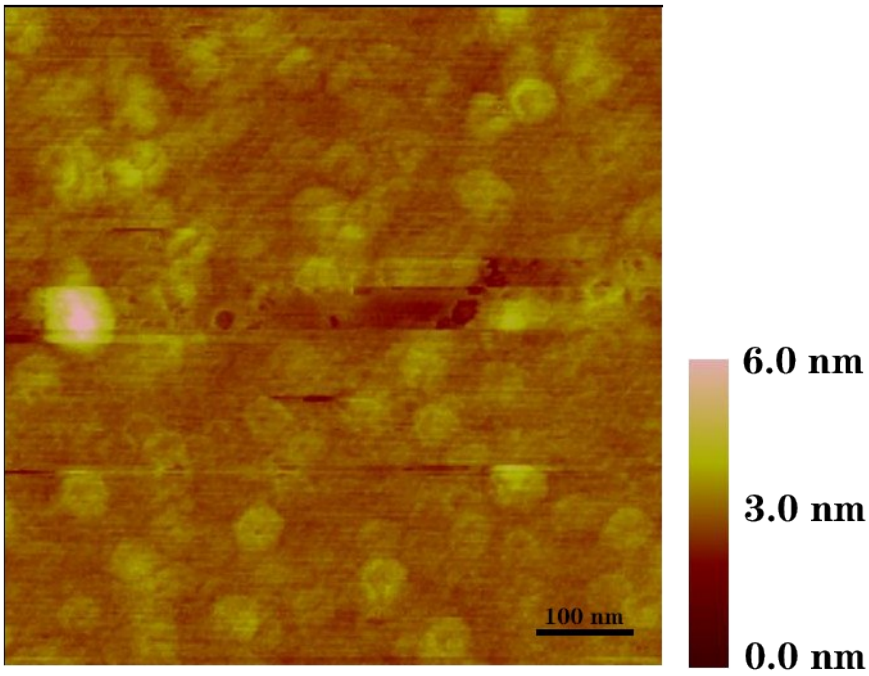

Figure S6 Representative zoom-out AFM image of DNA nanocage in open state

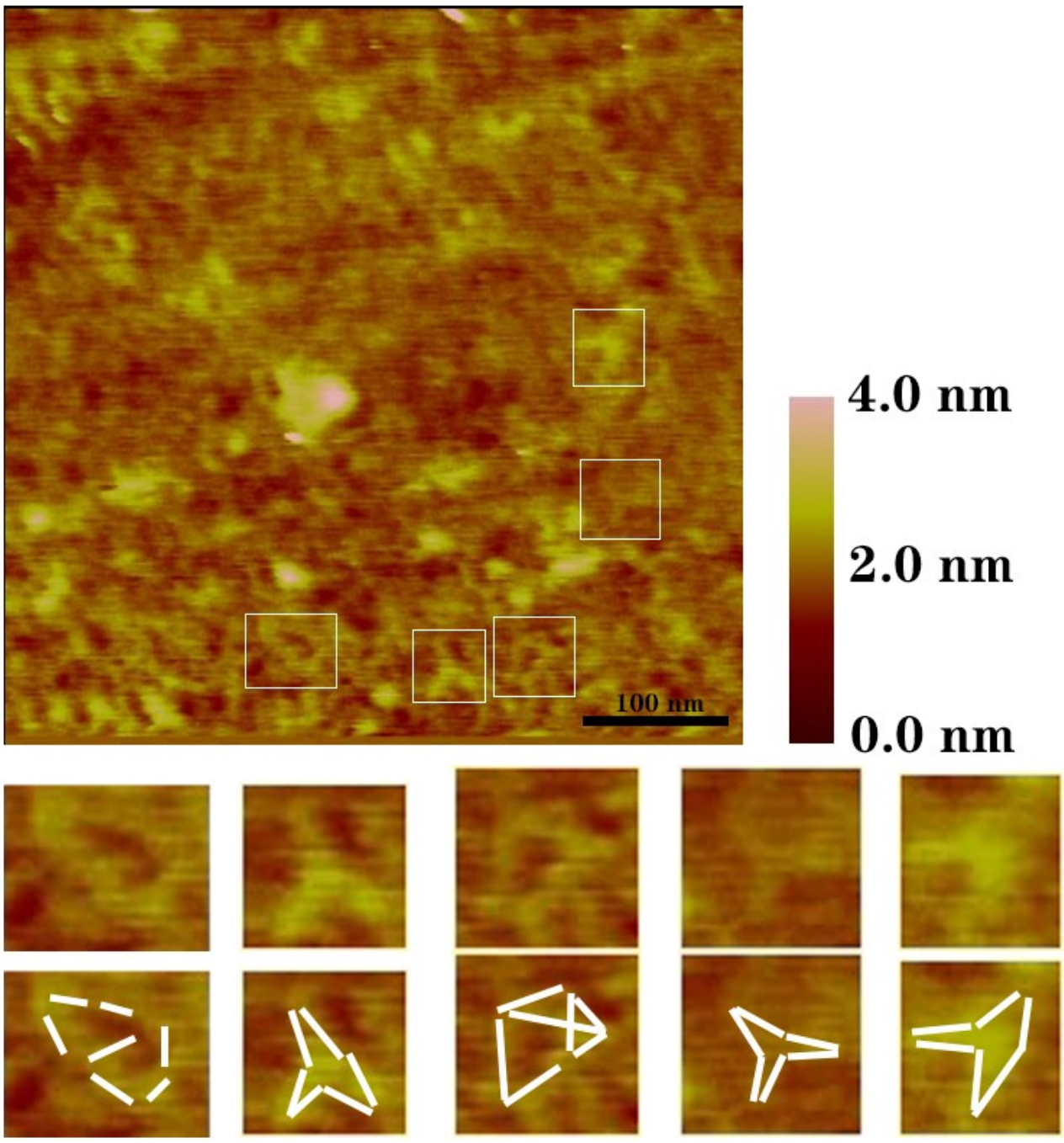

Figure S7 Representative zoom-out and zoom-in AFM image of DNA nanocage in closed state

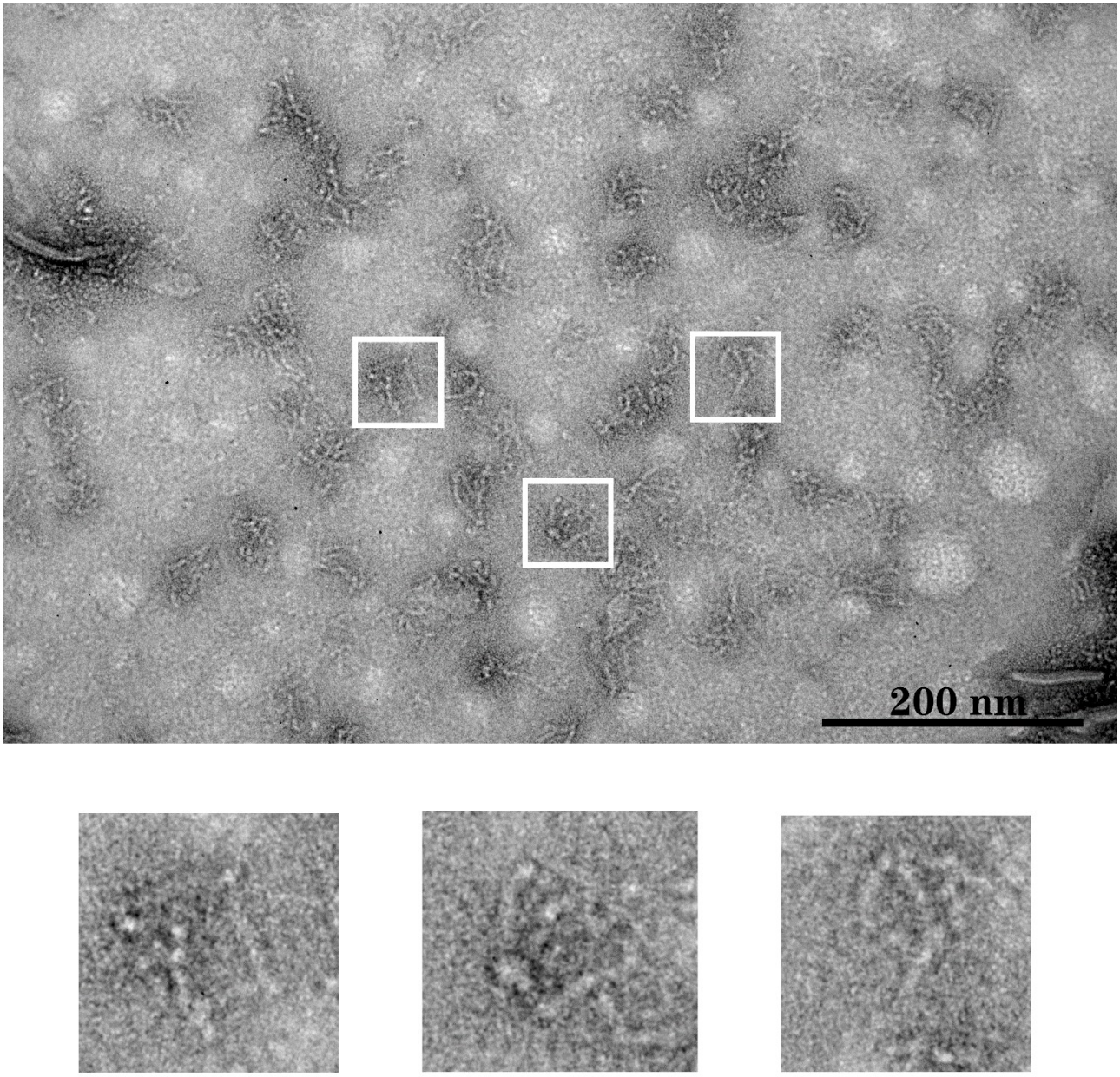

Figure S8 Representative zoom-out and zoom-in TEM image of DNA nanocage in open state

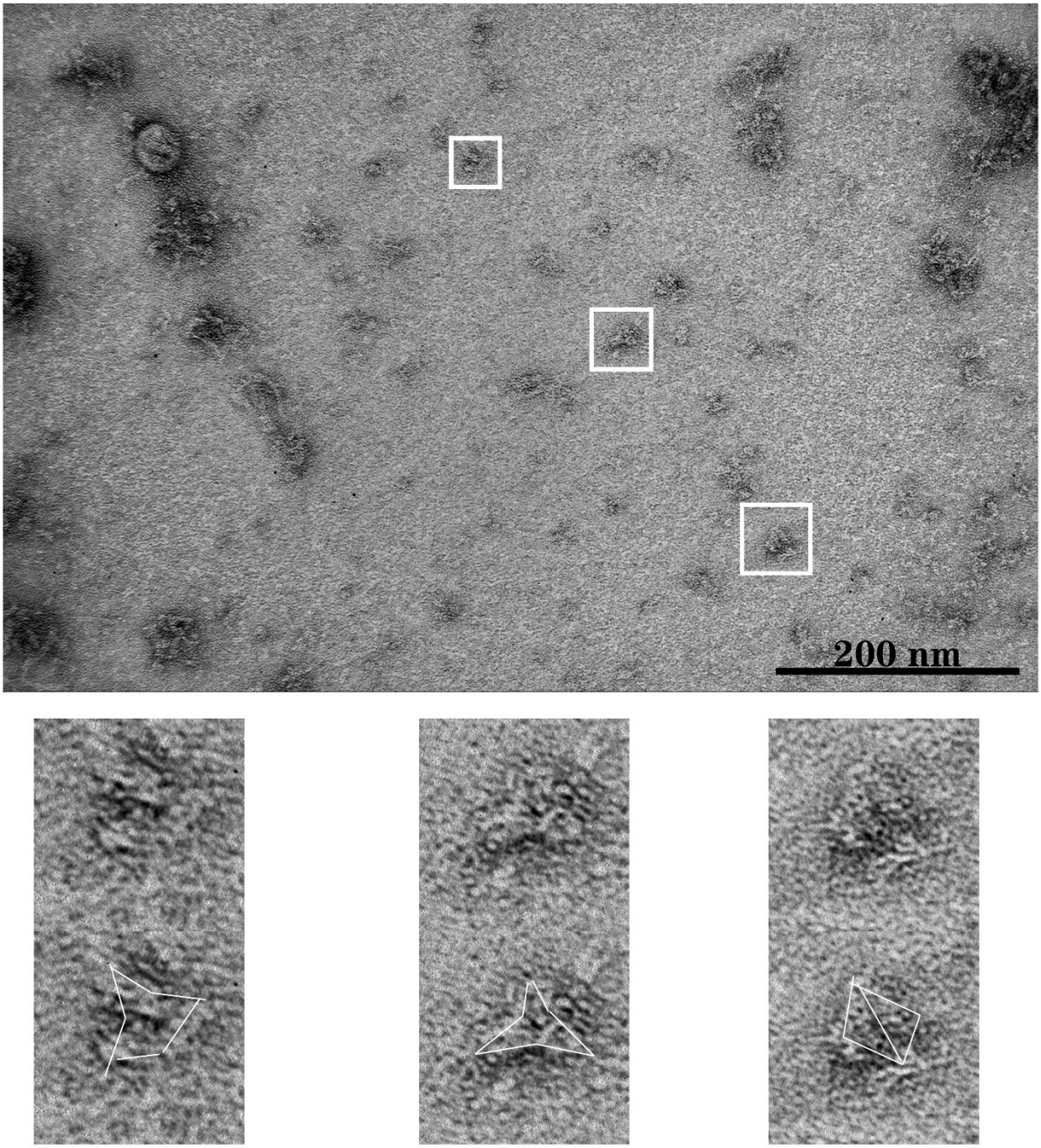

Figure S9 Representative zoom-out and zoom-in TEM image of DNA nanocage in closed state.

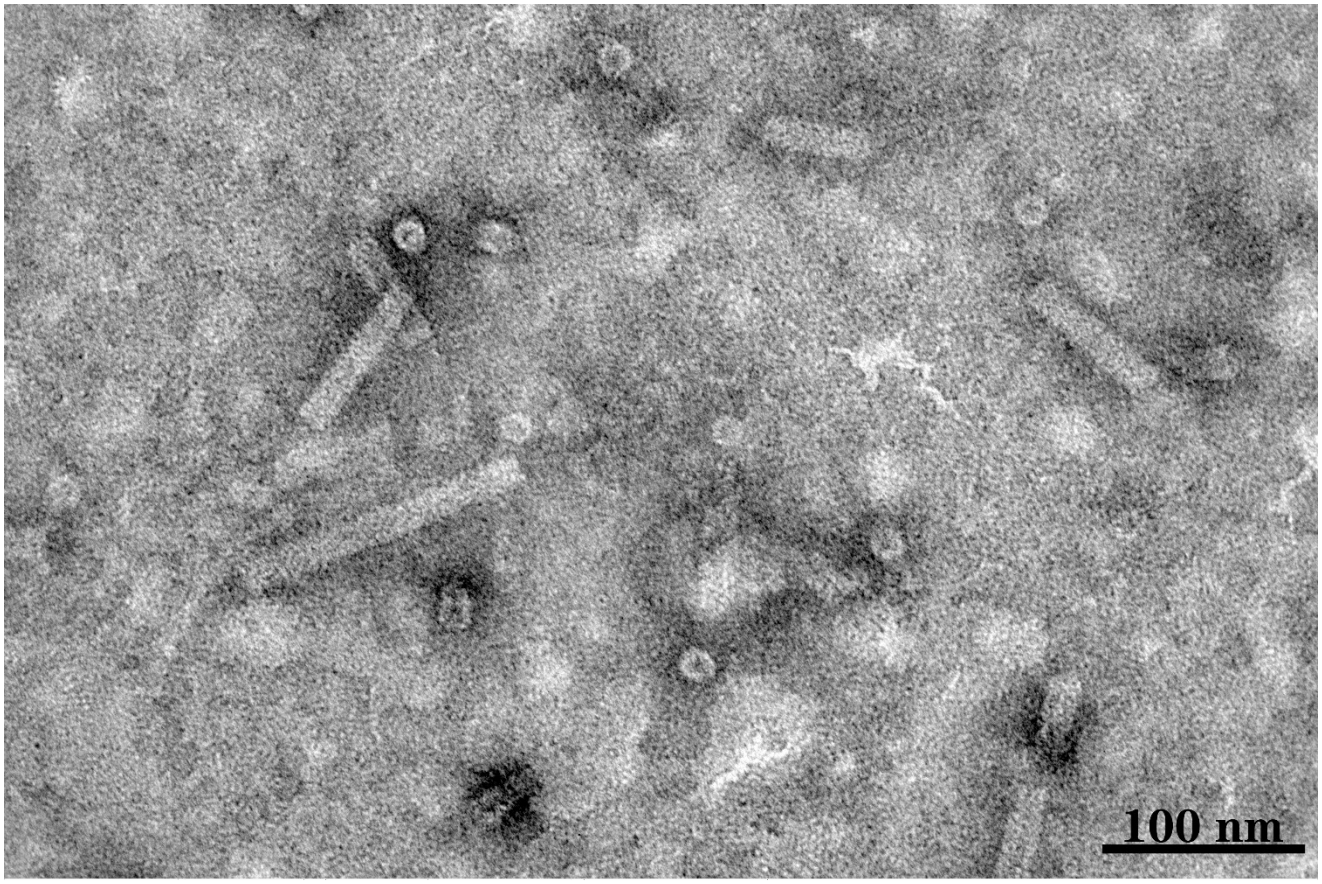

Figure S10 Representative zoom-out TEM image of TMV and TMV disks.

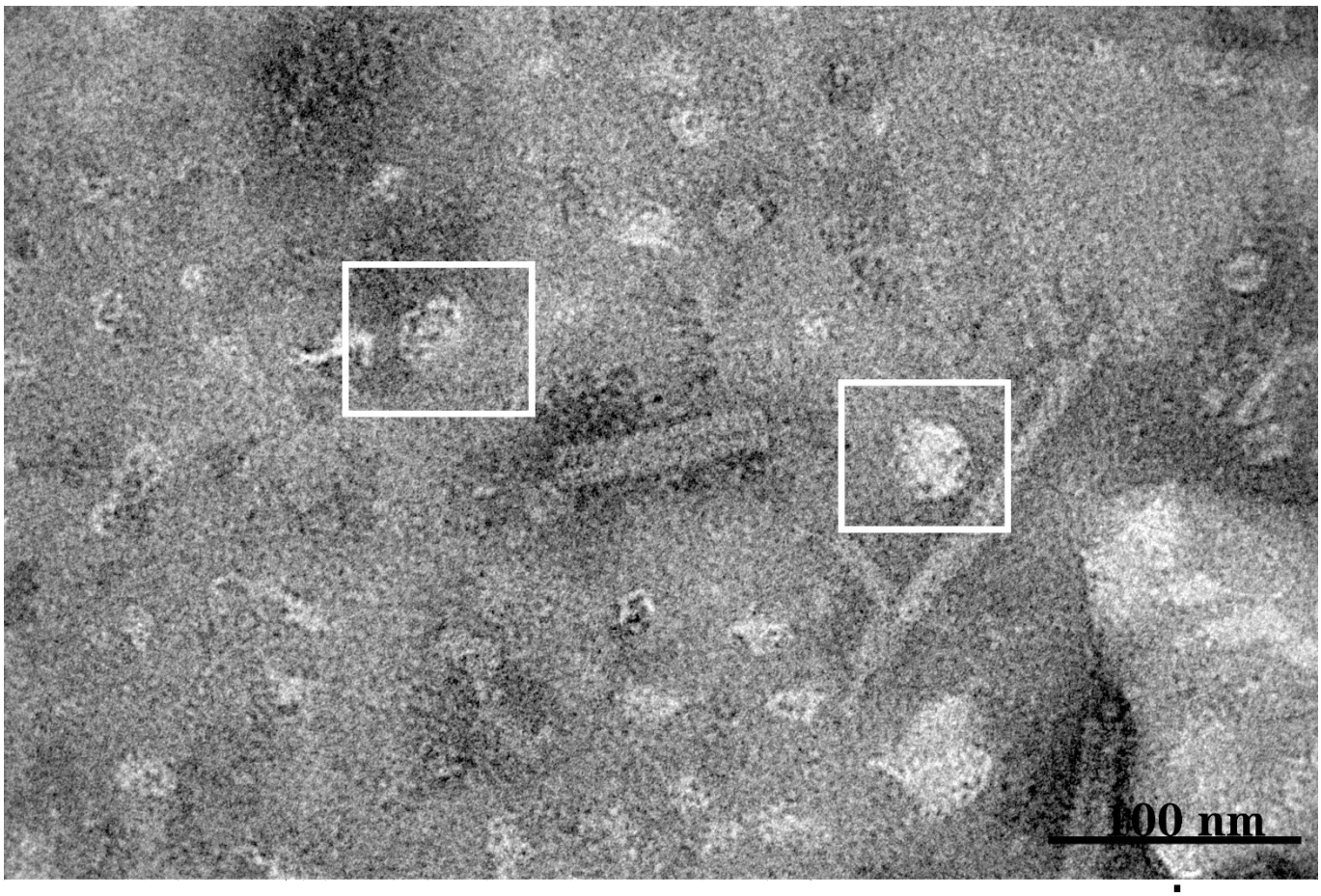

Figure S11 Representative zoom-out TEM image of TMV disk-nanocage complex in open state

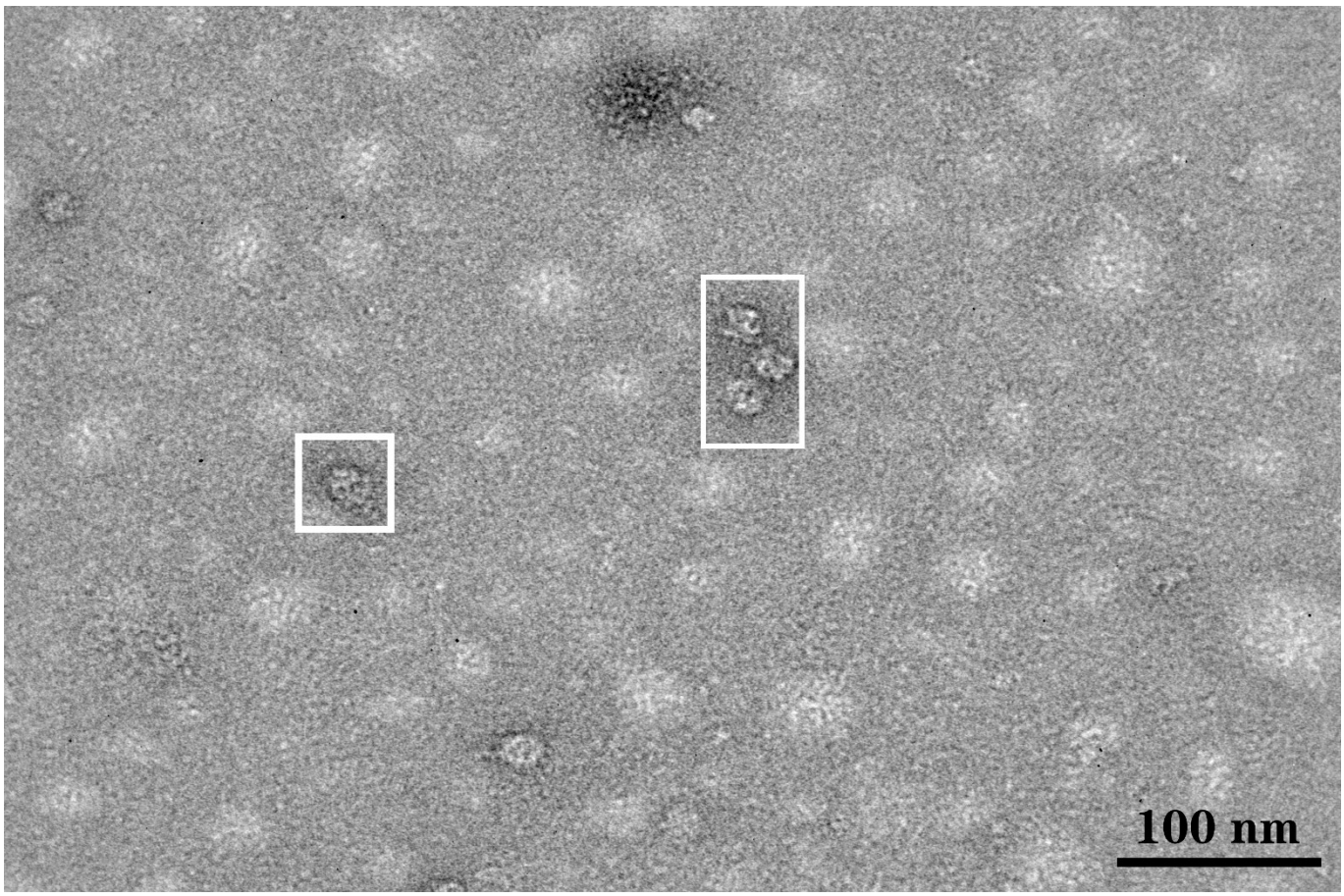

Figure S12 Representative zoom-out TEM image of TMV disk-nanocage complex in closed state

References for Supporting Information

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2 Ouldridge, T. E.; Louis, A. A.; Doye, J. P., Structural, mechanical, and thermodynamic properties of a coarse-grained DNA model. The Journal of chemical physics 2011, 134, 085101.