

Folding of Single-stranded Circular DNA into Rigid Rectangular DNA Accelerates its Cellular Uptake

Shozo Ohtsuki^{a,#}, Yukako Shiba^{a,#}, Tatsuoki Maezawa^a, Kumi Hidaka^b, Hiroshi Sugiyama^{b,c},
Masayuki Endo^{b,c}, Yuki Takahashi^a, Yoshinobu Takakura^a, Makiya Nishikawa^{a,d,*}

^a Department of Biopharmaceutics and Drug Metabolism, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan

^b Department of Chemistry, Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan

^c Institute for Integrated Cell-Material Sciences, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan

^d Laboratory of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Noda, Chiba 278-8510, Japan

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

These authors contributed equally.

* Address correspondence to Makiya Nishikawa, makiya@rs.tus.ac.jp

Supplementary Information

Supplementary Tables

Supplementary Table S1. Sequences of staples.

Name	Sequences (5' → 3')
0[68]1[52]	TTTGGGGTGAACATATTGACTGAGAGATTAA
0[100]1[84]	TAGAATGAGGTTGGCAAGTTAACAGACTTAC
*0[132]0[101]	TGTTATTGTCTTTGGGCTTCTATATACATT
0[164]1[148]	TTCTTCCTTACAGTCTGTTTC
0[188]1[180]	TAAATCTTGATATTACTCACTGT
1[53]3[52]	TTATTATGCTTAATGGAATTATAACAGCCAGT
1[85]3[84]	AGGTATCAAAAATACATATGATAAAATTAAGAT
1[117]2[101]	AGGTCACTTGTAAAATGCCATTCAAAATC
1[149]3[148]	TGAGCTCTCTAAAGAAAAAGTCTCCATT
1[181]3[180]	CTATCCCCATTACATATAAAATTGTGT
2[36]0[29]	TCAGGTCACAGAGAGCTGAACAAAATTCCATACCACATT
2[68]0[69]	TATGCACATGGGTAGTGTGATAAAAACAGCTT
2[100]1[116]	CTTAAGGATTCAATTAGTTCTTATTTCAT
2[132]0[133]	TTTCCCATTACATATATCAATGGGAATATT
2[164]0[165]	CCTGTTTACTCTATTATCAGCGTGGAAACA
3[53]5[52]	TCACATGCCTAAACCCCTCATGGCTAAGCTCTC
3[85]5[84]	CAGCAGTTTTAATGTACTAAGCAAATCAGC
3[117]4[101]	TCTAAAAGTGTGGCATGCTCTATGTACTAA
3[149]5[148]	TAGACTTAGTCATAAGAAATTTCATGGCC
3[181]5[180]	GTATGGTATAACAATCTTTCTTTTTAAAA
4[36]2[37]	AAGCTCTCCAGATGAAAACCAAGAAATATGTT

4[68]2[69] TTAATGAACCTAGAGATAATTAAATCTATCA
 4[100]3[116] GCTCTCATCAACCTGTTGATAGTATATTATT
 4[132]2[133] TGAAGTCTATTAAAGTTGCCATCAT
 4[164]2[165] GACTTGTATAATTCACTGGAATTATATTTC
 5[53]7[52] ATGTTGAGGTTCCCTAGGTTATCCAGGGCAA
 5[85]7[84] AACTAAAAAAAAGAATATATAAGGCAATCTCT
 5[117]6[101] GTGTTGCACATATTACAATTCTCTAACAGTTTA
 5[149]7[148] TGACTTTTACTGATTGTAAGACCAATGATA
 5[181]7[180] ATAAGTCTATCTGTTCAAATTTTTCTCATG
 6[36]4[37] TGCTCCTCTCATGTTCATGTACTAACATGTACT
 6[68]4[69] GGTTTAAACAATAAAATTAAATTCTCATGT
 6[100]5[116] TAAGAAAATAGCCTCTAACAGGTTTACTGGG
 6[132]4[133] ATAATCTGAATATTCTGTCAATTCTACAC
 6[164]4[165] TTATTTTCTTAATGGTTTTAACAGTGGTTAG
 7[53]9[52] ACTCTCTCAGTCCTCTCACCCACACAGGGTGA
 7[85]9[84] GTCATGGCCCACCTCACTCCACTCAGTCATCC
 7[117]8[101] TATTCTCCACAGATGTTACTTAGCTGAAGTT
 7[149]9[148] GTTCTCTGGTTAATTGTCAACTACACATCCC
 7[181]9[188] TGTAATGATGTATTACTATGCATGGTCAACTGGCCAT
 8[36]6[37] AGGGTGTTGCCTGCTGGTCTCTCTCAGTCC
 8[68]6[69] GCTCATCCCCCAGGGCTGCTCTCTTAA
 8[100]7[116] GGTGGAGAAGGGCCACTGGCATCCCCTTTAA
 8[132]6[133] AATTAAGCTAATTAGTGTATGTTCTTTT
 8[164]6[165] ATGCCAATATTCTGAGATTGAGTGTCTAGTT
 *9[53]8[69] CATCATCTCTGACCACACCTGCAAAGCATA
 ...

- *9[85]9[116] - TCCACAAAATCTCTGGAGAACCCAACCTGTC

- 9[117]8[133] - AGTCCAGAACTCAACAGCTCCAGCCCTGTTT

- *9[149]8[165] - TGGCTGTGAGCACTGGGACAGCACGATATACT

- 9[29] 8[37] - GGACTGCTGAGATGAACCCAAGCC

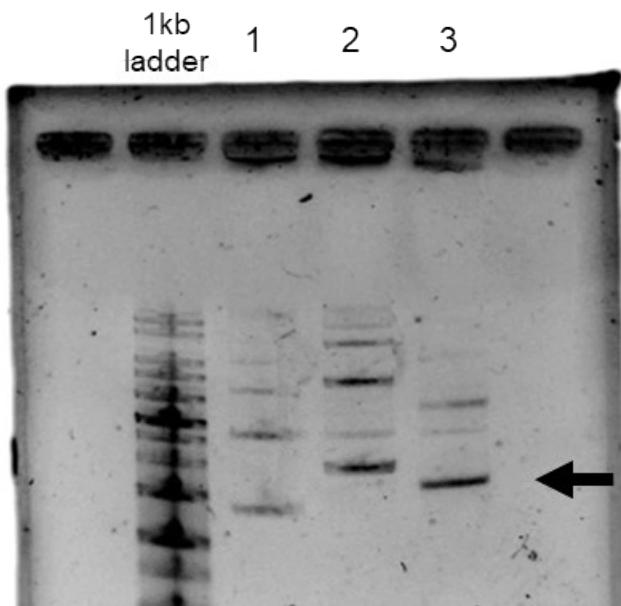
All ODNs have a phosphodiester backbone. Modification with Alexa Fluor 488 at the 5'-end is indicated by asterisk (*).

Supplementary Table S2. DNA nanostructures with excluded staples.

Nanostructure	Staples excluded
RecDNA	None
$\Delta 6c$	1[85]3[84], 3[85]5[84], 4[132]2[133], 5[85]7[84], 6[132]4[133], 8[132]6[133]
$\Delta 12c$	1[85]3[84], 1[149]3[148], 3[85]5[84], 3[149]5[148], 4[68]2[69], 4[132]2[133], 5[85]7[84], 5[149]7[148], 6[68]4[69], 6[132]4[133], 8[68]6[69], 8[132]6[133]
$\Delta 18c$	1[53]3[52], 1[85]3[84], 1[149]3[148], 3[53]5[52], 3[85]5[84], 3[149]5[148], 4[68]2[69], 4[132]2[133], 4[164]2[165], 5[53]7[52], 5[85]7[84], 5[149]7[148], 6[68]4[69], 6[132]4[133], 6[164]4[165], 8[68]6[69], 8[132]6[133], 8[164]6[165]
$\Delta 24$	1[53]3[52], 1[85]3[84], 1[149]3[148], 1[181]3[180], 3[53]5[52], 3[85]5[84], 3[149]5[148], 3[181]5[180], 4[36]2[37], 4[68]2[69], 4[132]2[133], 4[164]2[165], 5[53]7[52], 5[85]7[84], 5[149]7[148], 5[181]7[180], 6[36]4[37], 6[68]4[69], 6[132]4[133], 6[164]4[165], 8[36]6[37], 8[68]6[69], 8[132]6[133], 8[164]6[165]
$\Delta 6p$	1[181]3[180], 3[181]5[180], 4[36]2[37], 5[181]7[180], 6[36]4[37], 8[36]6[37],
$\Delta 12p$	1[53]3[52], 1[181]3[180], 3[53]5[52], 3[181]5[180], 4[36]2[37], 4[164]2[165], 5[53]7[52], 5[181]7[180], 6[36]4[37], 6[164]4[165], 8[36]6[37], 8[164]6[165]
$\Delta 18p$	1[53]3[52], 1[149]3[148], 1[181]3[180], 3[53]5[52], 3[149]5[148], 3[181]5[180], 4[36]2[37], 4[68]2[69], 4[164]2[165], 5[53]7[52], 5[149]7[148], 5[181]7[180], 6[36]4[37], 6[68]4[69], 6[164]4[165], 8[36]6[37], 8[68]6[69], 8[164]6[165]
Scaf4	Except for 0[132]0[101], 9[53]8[69], 9[85]9[116], 9[149]8[165]

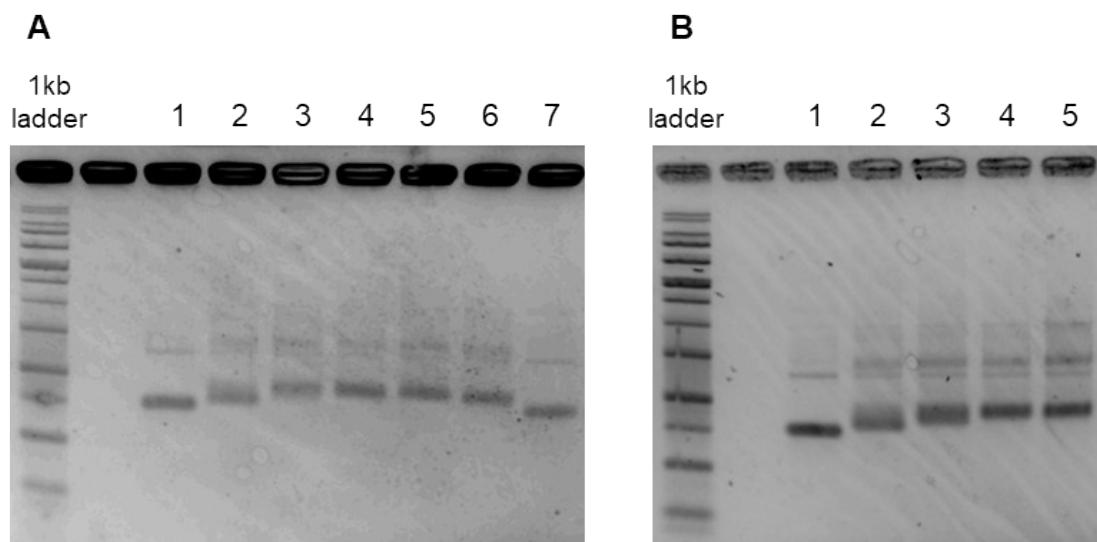
Supplementary Figures

Supplementary Figure S1



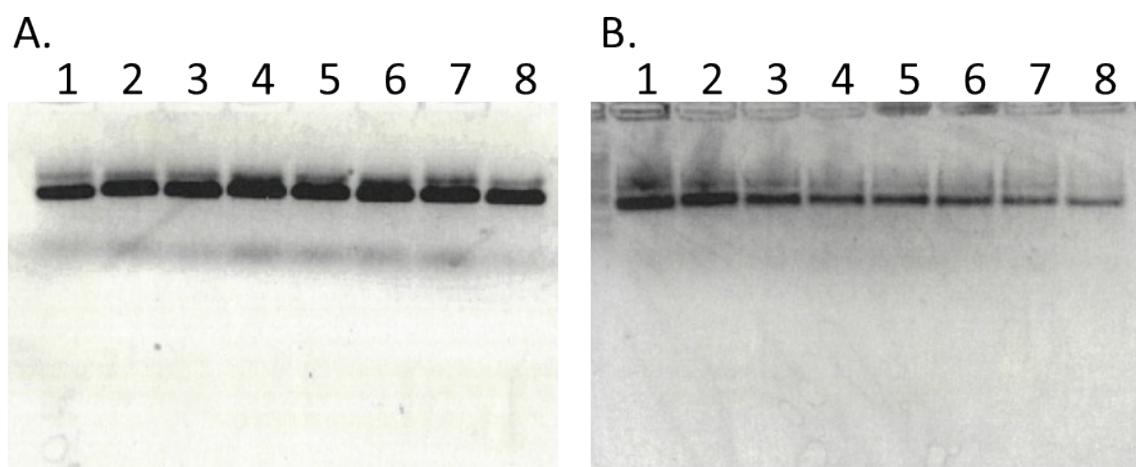
Supplementary Figure S1. Agarose gel electrophoresis analysis of scaffold preparation. Lane 1, double strand circular DNA; lane 2, nicked double strand circular DNA; lane 3, scaffold. Arrow indicates scaffold.

Supplementary Figure S2



Supplementary Figure S2. Agarose gel electrophoresis analysis of DNA nanostructures. A, lane1, scaffold; lane2, RecDNA; lane3, $\Delta 6c$; lane4, $\Delta 12c$; lane5, $\Delta 18c$; lane6, $\Delta 24$; lane7, Scaf4. B, lane1, scaffold; lane2, RecDNA; lane3, $\Delta 6p$; lane4, $\Delta 12p$; lane5, $\Delta 18p$.

Supplementary Figure S3



Supplementary Figure S3. Agarose gel electrophoresis analysis of DNA nanostructures. The fluorescence intensity of the bands of Alexa Fluor 488-labeled DNA nanostructures (A) and of EtBr staining of DNA (B). Lane1, RecDNA; lane2, Δ 6c; lane3, Δ 6p; lane4, Δ 12c; lane5, Δ 12p; lane6, Δ 18c; lane7, Δ 18p; lane8, Δ 24.