Electronic Supplementary Information

Positive supercoiling affilated with nucleosome formation repairs

non-B DNA structures

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EXTENDED EXPERIMENTAL PROCEDURES

Detailed experimental descriptions of Fig. 2D: (D) Gel-shift analysis of G-quadruplex reparation. Lane M: Molecular weight markers; Lane 1: DNA 1 alone (see Fig. 2A for its structure and Tab. S5 for its detailed nucleotide sequence); Lane 2: Nuclesomes formed between DNA 1 and histone octamers (Structure 1 in Fig. 2A). Experimental procedures: Preparation of nucleosomes from DNA 1 and histone proteins was carried out following reported procedures¹. More specifically, a mixture (20 µl) containing ~5 pmol DNA 1, 20 µM recombinant human Histone H2A/H2B Dimer (New England Biolabs), 10 µM Histone H3.1/H4 Tetramer (New England Biolabs) and 2 M NaCl was kept at room temperature for 30 minutes. NaCl in the mixture was diluted next with dilution buffer (10 mM Tris at pH 8.0) for four times to concentrations of 1.48 M, 1.00 M, 0.60 M, and 0.25 M respectively. The procedures to determine the concentration of binding sites in DNA follow the instructions of EpiMark[™] Nucleosome Assembly Kit provided by New England Biolabs (Ipswich, MA). The ratio of concentration of binding site in DNA to histone octamer was set to be 1:1.5, which ensured that the saturated nucleosomes can be formed within the target DNA. After each dilution, the resultant mixtures were further kept at room temperature for 30 minutes. The mixture obtained from the final step of dilutions was then used for electrophoretic analysis; Lane 3: A mixture obtained after the resultant nucleosomes were digested by proteinase K. Experimental procedures: A mixture containing 0.2 units of proteinase K and 10 µl of the previously obtained nucleosome solution (the same batch of solution as the one loaded into Lane 2 in Fig. 2B) was incubated at 37 °C for 1 hour followed by the electrophoretic analysis; Lane 4: A

mixture obtained after the resultant nuclesomes were digested by proteinase K followed by DNA relaxation through the action of Topo I. *Experimental procedures*: A solution containing 20 units of Topo I, 1 x Topo I buffer (50 mM Tris-HCl at pH 8.0, 150 mM NaCl, 10 mM, MgCl₂, 5 mM ATP, 0.5 mM dithiothreitol, 0.1% BSA) and 10 μ l of the same batch of mixture as the one loaded into Lane 3 was incubated at 37 °C for 1 hour followed by the electrophoretic analysis.

Detailed experimental descriptions of Fig. 3D: (D) Gel-shift analysis of cruciform reparation. Lane M: Molecular weight markers; Lane 1: DNA 3 alone (see Fig. 3A for its structure and Tab. S9 for its detailed nucleotide sequence); Lane 2: Nucleosomes formed between DNA 3 and histone octamers (Structure 1 in Fig. 3A). Experimental procedures: Preparation of nucleosomes from DNA 3 and histone proteins was carried out following reported procedures¹. More specifically, a mixture (20 μ l) containing ~5 pmol DNA 3, 20 µM recombinant human Histone H2A/H2B Dimer (New England Biolabs), 10 µM Histone H3.1/H4 Tetramer (New England Biolabs) and 2 M NaCl was kept at room temperature for 30 minutes. NaCl in this mixture was diluted next with dilution buffer (10 mM Tris at pH 8.0) for four times to concentrations of 1.48 M, 1.00 M, 0.60 M, and 0.25 M respectively. The procedures to determine the concentration of binding sites in DNA follow the instructions of EpiMark[™] Nucleosome Assembly Kit provided by New England Biolabs (Ipswich, MA). The ratio of concentration of binding site in DNA to histone octamer was set to be 1:1.5, which ensured that the saturated nucleosomes can be formed within the target DNA. It has been well established that 146 base pairs (bp) of DNA can wrap around one

histone octamer to form a nucleosome core and each nucleosome is connected to its neighbours by a short segment of linker DNA (mean value is 45 bp in length)^{7,8}. Since the duplex region of cruciform containing circular DNA (DNA 3) in our studies is 1185 bp in length, ~6 nucleosomes (1185/191 = 6.2) can be formed within the circular DNA on average. After each dilution, the resultant mixtures were further kept at room temperature for 30 minutes. The mixture obtained from the last final of dilutions was then used for electrophoretic analysis; Lane 3: A mixture obtained after the resultant nucleosomes were digested by proteinase K. *Experimental procedures*: A mixture containing 0.2 units of proteinase K and 10 µl of the previously obtained nucleosome solution (the same batch of solution as the one loaded into Lane 2 in Fig. 3D) was incubated at 37 \mathbb{C} for 1 hour followed by the electrophoretic analysis.

Detailed experimental descriptions of Fig. 4D: (D) Gel-shift analysis of PNA-caused non-B structure reparation. Lane M: Molecular weight markers; Lane 1: DNA 5 alone (see Fig. 4A for its structure and Tab. S13 for its detailed nucleotide sequence); Lane 2: Nuclesomes formed between DNA 5 and histone octamers. *Experimental procedures*: Preparation of nucleosomes from DNA 5 and histone proteins was carried out following reported procedures¹. More specifically, a mixture (20 μ l) containing ~5 pmol DNA 5, 20 μ M recombinant human Histone H2A/H2B Dimer (New England Biolabs), 10 μ M Histone H3.1/H4 Tetramer (New England Biolabs) and 2 M NaCl was kept at room temperature for 30 minutes. NaCl in the mixture was diluted next with dilution buffer (10 mM Tris at pH 8.0) for four times to concentrations of 1.48 M, 1.00 M, 0.60 M, and 0.25 M respectively. The procedures

to determine the concentration of binding sites in DNA follow the instructions of EpiMark[™] Nucleosome Assembly Kit provided by New England Biolabs (Ipswich, MA). The ratio of concentration of binding site in DNA to histone octamer was set to be 1:1.5, which ensured that the saturated nucleosomes can be formed within the target DNA. After each dilution, the resultant mixtures were further kept at room temperature for 30 minutes. The mixture obtained from the final step of dilutions was then used for electrophoretic analysis; Lane 3: A mixture obtained after the resultant nucleosomes were digested by proteinase K. Experimental procedures: A mixture containing 0.2 units of proteinase K and 10 µl of the previously obtained nucleosome solution (the same batch of solution as the one loaded into Lane 2 in Fig. 4B) was incubated at 37 °C for 1 hour followed by the electrophoretic analysis; Lane 4: A mixture obtained after the resultant nuclesomes were digested by proteinase K followed by DNA relaxation through the action of Topo I. Experimental procedures: A solution containing 20 units of Topo I, 1 x Topo I buffer (50 mM Tris-HCl at pH 8.0, 150 mM NaCl, 10 mM, MgCl₂, 5 mM ATP, 0.5 mM dithiothreitol, 0.1% BSA) and 10 µl of the same batch of mixture as the one loaded into Lane 3 in Fig. 4D was incubated at 37 °C for 1 hour followed by the electrophoretic analysis.

Experimental procedures for DNA sample preparations and AFM examination: All micas used in the current studies were modified on their surfaces with (3-aminopropyl)triethoxysilane (APS-micas) following reported procedures². DNA samples for AFM examination were prepared into solutions at first that contained 20 mM Tris-HCl (pH = 7) and 0.1 to 0.01 μ g/ml DNA. 5 μ l to 10 μ l of those DNA

solutions were placed next in the middles of the newly prepared APS-mica plates (1 x 1 cm²), which were further kept at room temperature for 5 minutes. The surfaces of the APS-mica plates bound by DNA were then rinsed using distilled water for 3 times. AFM images of DNA molecules on the APS-mica plates were obtained in Tapping ModeTM on a MultimodeTM AFM (Veeco, Santa Barbara, CA) in connection with a Nanoscope VTM controller. Antimony (n) doped Si cantilevers with nominal spring constants between 20 and 80 N/m were selected. Scan frequency was 1.9 Hz per line and the modulation amplitude was in a nanometer range. All DNA sample determinations were carried out in air at room temperature.

In the case of Holliday junction (cruciform) and spurs, the observed shapes were significantly different from anything seen on pure duplex DNA. As a result, all of these structures were included in the dataset. Since variations in the imaging surface and/or kinks in the circular DNA, small raised structures (blobs) were occasionally seen on pure duplex DNA. To distinguish the newly formed non-B structures (e.g. PNA-DNA binding complex) from the features occasionally found on the pure duplex DNA, a criterion was set according to previous the studies.⁶ The normal height and the peak height were determined for 20 duplex DNA molecules. The mean of normal height was 0.53 ± 0.01 nm, and the mean of peak height was 0.68 ± 0.02 nm, with a highest absolute value of 0.86 nm. Consequently, any blob < 0.9 nm in height was excluded from the dataset and any blob ≥ 1 nm was included. The height measurements were taken across the middle of each blob. Frequency distributions of lengths (in nm) of DNA were obtained by detecting the circumference alone the

backbone of circular DNA, which were measured by drawing a series of very short lines along the DNA contour and summating the lengths.⁶



Fig. S1 Synthesis and structural confirmation of DNA 1.

(A) Schematic illustration of our synthetic route towards DNA 1. (B) Electrophoretic analysis of DNA products involved in synthesizing DNA S3 (see Fig. S1A). Lane M: molecular weight markers; Lane 1: DNA S1 generated through PCR amplification reactions (Step 1); Lane 2: DNA S2 with its cohesive ends created through using SacI (Step 2); Lane 3: crude product of DNA S3 produced through reaction of DNA S2 and T4 DNA ligase; Lane 4: pure DNA S3 obtained through hydrolysis of crude product of DNA S3 by Nuclease BAL-31 (Step 3). (C) Structural confirmation of DNA S3 using AFM. Left: AFM images of DNA S3. The DNA sample used for this AFM examination was the same batch of sample as the one loaded into Lane 4 in Fig. S1B. Right: section analyses of DNA S3. The backbone circularity of DNA S3 was

verifiable by the naked eyes and section analyses. (D) Electrophoretic analysis of DNA products involved in synthesizing DNA 1 (see Fig. S1A). Lane M: molecular weight markers; Lane 1: DNA S3; Lane 2: A nick-containing circular DNA (DNA S4) created through using Nt.BsmAI (Step 4); Lane 3: A nicked site- and G-quadruplex-containing circular DNA (DNA S5) obtained by incubation of DNA S4 in 40% PEG200 at 95 °C for 5 minutes followed by cooling the mixture to room temperature; Lane 4: DNA 1 obtained by incutation of DNA S5 with T4 DNA ligase. (E) Structural confirmation of DNA 1 using AFM. Left: AFM images of DNA 1. The DNA sample used for this AFM examination was the same batch of sample as the one loaded into Lane 4 in Fig. S1D. Right: section analyses of DNA 1. The presence of G-quadruplex in DNA 1 and its backbone circularity were verifiable by the naked eyes and section analyses.

Experimental procedures:

Step 1: X2420G (plasmid DNA) was purchased from Generay Biotech (Shanghai, China). The forward primer (Primer 1 in Tab. S1) contained the cytosine-rich segment (-CCCCAAAAACCCCAAAAACCCCAAAAACCCCAAAA-). The detailed nucleotide sequences of Forward Primer (Primer 1) and Reverse Primer (Primer 2) used in the current studies are shown in Tab. S1. The PCR amplification reactions were carried out following reported procedures³⁻⁴ and the amplification product (DNA S1 whose sequence is shown in Tab. S2) was verified through electrophoresis (lower band in Lane 1 in Fig. S1B).

Step 2: A mixture containing 10 mM Bis-Tris-Propane-HCl, 10 mM MgCl₂, 1 mM

Dithiothreitol, 10 units SacI and ~2 μ g DNA S1 was incubated at 37 °C for 1 hour, which gave rise to a cohesive end-containing linear DNA (DNA S2 whose sequence is shown in Tab. S3; also see Lane 2 in Fig. S1B).

Step 3: A mixture containing 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM ATP, 10 mM dithiothreitol, 20 units T4 DNA ligase and ~500 ng DNA S2 was incubated at 16 °C for 8 hours (Lane 3 in Fig. S1B). The resultant reaction mixture was allowed next to react with BAL-31 (an exonuclease that hydrolyzes opening end-containing DNA) in order to acquire pure closed circular DNA products (DNA S3 whose sequence is shown in Tab. S4, also see Lane 4 in Fig. S1B).

Step 4: A mixture containing 5 units of Nt.BsmAI, 1 x Nt.BsmAI buffer (20 mM Tris-acetate, 50 mM potassium acetate, 10 mM Magnesium Acetate, 1 mM Dithiothreitol) and ~500 ng DNA S3 was incubated at 37 °C for 1 hour to generate a nick-containing circular DNA (DNA S4; also see Lane 2 in Fig. S1D).

Step 5: A mixture containing 20 mM Tris-HCl (PH = 7), 40% PEG 200, 150 mM KCl and ~500 ng DNA S4 was kept at 95 °C for 5 minutes followed by cooling the mixture to room temperature to produce both nicked site- and G-quadruplex-containing circular DNA⁵ (DNA S5; also see Lane 3 in Fig. S1D).

Step 6: A mixture containing 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM ATP, 10 mM dithiothreitol, 5 units T4 DNA ligase and ~500 ng DNA S5 was incubated at 25 $^{\circ}$ C for 2 hours to generate our desired G-quadruplex-containing circular DNA (DNA 1 whose sequence is shown in Tab. S5, also see Lane 4 in Fig. S1D).



Fig. S2 Examination of effects of buffer and salts on the stability of the G-quadruplex residing in a circular DNA (DNA 1) (the tests conducted in the current section served as the control experiments for those shown in Fig. 2).

(A) Agarose gel electrophoretic analysis of our control studies Lane M: Molecular weight markers; Lane 1 to Lane 4: The samples loaded into the four lanes were prepared in the same ways as for those loaded into Lane 1 to Lane 4 in Fig. 2D except that hostone octamers were not used in the control experiments. (B) Left: AFM images of the final products of our current control studies. The DNA used for this AFM examination was the same batch of sample as the one loaded into Lane 4 in Fig S2A. Right: Section analyses of one of the AFM images obtained from our current control studied. The presence of G-quadruplex in the final DNA product was verifiable by the naked eyes (left) and section analyses (right). (C) Frequency distributions of the lengths (nm) of the final products of our current control studies. The analyses shown in this section were conducted through following literature reported procedures.⁶



Fig. S3 Synthesis and structural confirmation of DNA 3.

(A) Schematic illustration of our synthetic route towards DNA 3. (B) Electrophoretic analysis of DNA products involved in synthsizing DNA S8 (see Fig. S3A). Lane M: molecular weight markers; Lane 1: DNA S6 generated through PCR amplification reactions (Step 1); Lane 2: DNA S7 with its cohesive ends created through using SacI (Step 2); Lane 3: crude product of DNA S8 produced through reaction of DNA S2 and T4 DNA ligase; Lane 4: pure DNA S8 obtained through hydrolysis of crude product of DNA S8 by Nuclease BAL-31 (Step 3) (C) Structural confirmation of DNA S8 using AFM. The sample used for this AFM examination was the same batch of sample as the one loaded into Lane 4 in Fig. S3B. The backbone circularity of DNA S8 was verifiable by the naked eyes. (D) Electrophoretic analysis of DNA products involved in synthesizing DNA 3 (see Fig. S3A). Lane M: molecular weight markers; Lane 1: DNA S9 obtained by incubation of DNA S8 with DNA gyrase; Lane 2: cruciform-containing circular DNA (DNA 3) obtain by incubation of DNA S8 with

100 mM NaCl overnight. (E) Structural confirmation of DNA S9 using AFM. The sample used for this AFM examination was the same batch of sample as the one loaded into Lane 2 in Fig. S3D. The presence of negative supercoiling in DNA S9 was verifiable by the naked eyes. (F) Structural confirmation of DNA 3 using AFM. The sample used for AFM examination was the same batch of sample as the one loaded into Lane 2 in Fig. S3D. The presence of cruciform in DNA 3 can be verifiable by the naked eyes.

Experimental procedures:

Step 1: X4510E (a plasmid DNA) was purchased from Generay Biotech (Shanghai, China). Nucleotide sequences of both Forward Primer (Primer 3) and Reverse Primer (Primer 4) are shown in Tab. S1. The PCR amplification reactions were carried out following reported procedures³⁻⁴ and the amplification products (DNA S6 whose sequence is shown in Tab. S6) were verified through electrophoresis (Lane 1 in Fig. S3B).

Step 2: A mixture containing 10 mM Bis-Tris-Propane-HCl, 10 mM MgCl₂, 1 mM Dithiothreitol, 10 units SacI and ~2 μ g DNA S6 was incubated at 37 °C for 1 hour to generate a cohesive end-containing linear DNA (DNA S7 whose sequence is shown in Tab. S7, also see Lane 2 in Fig. S3B).

Step 3: A mixture containing 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM ATP, 10 mM dithiothreitol, 20 units T4 DNA ligase and ~500 ng DNA S7 was incubated at 16 % for 8 hours (Lane 3 in Fig. S3B). The resultant reaction mixture was allowed next to react with BAL-31 (an exonuclease that hydrolyzes open end-containing DNA)

in order to acquire pure closed circular DNA products (DNA S8 whose sequence is shown in Tab. S8, also see Lane 4 in Fig. S3B).

Step 4: A mixture containing 5 units of DNA gyrase, 1 x DNA gyrase buffer (35 mM Tris-HCl, 24 mM KCl, 4 mM MgCl₂, 2 mM DTT, 1.75 mM ATP, 5 mM spermidine, 0.1 mg/ml BSA and 6.5% Glycerol) and ~500 ng DNA S8 was incubated at 37 °C for 1 hour to generate negatively supercoiled DNA (DNA S9; also see Lane 1 in Fig. S3D).

Step 5: A mixture containing 20 mM Tris-HCl (PH = 7), 100 mM NaCl and ~500 ng DNA S9 was kept at room temperature overnight to produce cruciform-containing circular DNA (DNA 3 whose sequence is shown in Tab. S9, also see Lane 2 in Fig. S3D).



Fig. S4 Examination of effects of buffer and salts on the stability of the cruciform residing in a circular DNA (DNA 3) (the tests conducted in the current section served as the control experiments for those shown in Fig. 3).

(A) Agarose gel electrophoretic analysis of our control studies Lane M: Molecular weight markers; Lane 1 to Lane 3: The samples loaded into the four lanes were prepared in the same ways as for those loaded into Lane 1 to Lane 3 in Fig. 3D except that hostone octamers were not used in the control experiments. (B) AFM images of the final products of our current control studies. The DNA sample used for this AFM examination was the same batch of sample as the one loaded into Lane 3 in Fig. S4A. The presence of cruciform in the final products obtained from our control studies was verifiable by the naked eyes. (C) Statistical analysis of DNA 3 and the final products of our current control studies in a $2 \times 2 \mu m^2$ area.



Fig. S5 Synthesis and structural confirmation of DNA 5.

(A) Schematic illustration of our synthetic route towards DNA 5. (B) Electrophoretic analysis of DNA products involved in synthsizing DNA S12 and DNA 5. Lane M: molecular weight markers; Lane 1: DNA S10 generated through PCR amplification reactions (Step 1); Lane 2: DNA S11 with its cohesive ends created through using SacI (Step 2); Lane 3: crude product of DNA S12 produced through reaction of DNA S2 and T4 DNA ligase; Lane 4: pure DNA S12 obtained through hydrolysis of crude product of DNA S12 by Nuclease BAL-31 (Step 3); Lane 5: A nick-containing circular DNA (DNA S13) created through using Nt.BsmAI (Step 4); Lane 6: PNA-DNA complex (DNA S14) obtained by PNA invasion (Step 5); Lane 7: DNA 5 obtained by ligase reaction (Step 6). (C) Structural confirmation of DNA S12 using AFM. Left: AFM images of DNA S12. The sample used for this AFM examination

was the same batch of sample as the one loaded into Lane 4 in Fig. S5B. Right: section analyses of DNA S12. The backbone circularity of DNA S12 was verifiable by the naked eyes and section analyses. (D) Structural confirmation of DNA 5 using AFM. Left: AFM images of DNA 5. The sample used for this AFM examination was the same batch of sample as the one loaded into Lane 7 in Fig. S5B. Right: section analyses of DNA 5. The presence of PNA-caused non-B structure in DNA 5 and its backbone circularity were verifiable by both naked eyes and section analyses.

Experimental procedures:

Step 1: X2420G (a plasmid DNA) was purchased from Generay Biotech (Shanghai, China) and used as the template for PCR amplification. Nucleotide sequences of Forward Primer (Primer 5) and Reverse Primer (Primer 6) are shown in Tab. S1. PCR amplification reactions were carried out through following reported procedures³⁻⁴ and the amplification products (DNA S10 whose sequence is shown in Tab. S10) were examined through electrophoresis (Lane 1 in Fig. S5B).

Step 2: A mixture containing 10 mM Bis-Tris-Propane-HCl, 10 mM MgCl₂, 1 mM Dithiothreitol, 10 units SacI and ~2 μ g DNA S10 was incubated at 37 °C for 1 hour to generate a cohesive end-containing linear DNA (DNA S11 whose sequence is shown in Tab. S11, also see Lane 2 in Fig. S5B).

Step 3: A mixture containing 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM ATP, 10 mM dithiothreitol, 20 units T4 DNA ligase and ~500 ng DNA S2 was incubated at 16 $^{\circ}$ C for 8 hours (Lane 3 in Fig. S5B). The resultant reaction mixture was allowed next to react with BAL-31 in order to acquire pure closed circular DNA products

(DNA S12 whose sequence is shown in Tab. S12, also see Lane 4 in Fig. S5B).

Step 4: A mixture containing 5 units of Nt.BsmAI, 1 x Nt.BsmAI buffer (20 mM Tris-acetate, 50 mM potassium acetate, 10 mM Magnesium Acetate, 1 mM Dithiothreitol) and ~500 ng DNA S12 was incubated at 37 °C for 1 hour to generate a nick-containing DNA (DNA S13; also see Lane 5 in Fig. S5B).

Step 5: A mixture containing 10mM Sodium-Phosphate (pH = 7), 0.1 μ M PNA 1 (see Fig. S7 for the structure of PNA 1) and ~500 ng DNA S13 was kept at 37 °C for 2 hours to produce both nicked site- and PNA-containing circular DNA (DNA S14, also see Lane 6 in Fig. S5B).

Step 6: A mixture containing 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM ATP, 10 mM dithiothreitol, 5 units T4 DNA ligase and ~500 ng DNA S13 was incubated at 25 $^{\circ}$ C for 2 hours to generate the desired PNA-containing circular DNA (DNA 5 whose sequence is shown in Tab. S13, also see Lane 7 in Fig. S5B).



Fig. S6 Examination of effects of buffer and salts on the stability of the PNA-DNA complex residing in a circular DNA (DNA 5) (the tests conducted in the current section served as the control experiments for those shown in Fig. 7).

(A) Agarose gel electrophoretic analysis of our control studies Lane M: Molecular weight markers; Lane 1 to Lane 4: The samples loaded into the four lanes were prepared in the same ways as for those loaded into Lane 1 to Lane 4 in Fig. 4D except that hostone octamers were not used in the control experiments. (B) Left: AFM image of the final products of our control studies. The DNA sample used for this AFM examination was the same batch of sample as the one loaded into Lane 4 in Fig. S6A. Right: Section analyses of one of the AFM images obtained from our control studied. The presence of PNA-caused non-B structure in the final DNA product was verifiable by the naked eyes (left) and section analyses (right). (C) Statistical analysis of DNA 5 and the final products of our current control studies in a 2 x 2 μ m² area.



Fig. S7 Structure of PNA 1 that was used in our studies.

This PNA is written staring from the N terminus to the C terminus following the conventions for regular peptide expression in which:

H: free amino group;

NH₂: terminal carboxamide;

Lys: lysine residue;

J: pseudoisocytosine;

eg1: 8-amino-3,6-dioxaoctanoic acid (linker unit).



Fig. S8 Examination of the disintegration of G-quadruplex in DNA 7 and DNA 9 during the course of nucleosome formation.

(A) Left: AFM image of the DNA 7. The DNA sample used for this AFM examination was synthesized in the same way shown in Fig. S1. The sequence of DNA 7 was shown in Tab. 14. Right: Section analyses of one of an AFM image obtained from Fig. 8A. The presence of G-quadruplex structures was verifiable by the naked eyes (left) and section analyses (right). (B) Left: AFM image of the DNA 8. The DNA sample used for this AFM examination was were prepared in the same ways as for those loaded into Lane 1 to Lane 4 in Fig. 2D except that DNA 7 were not used as the substrate. Right: Section analyses of one of an AFM image obtained from Fig. 8B. The absence of G-quadruplex structures was verifiable by the naked eyes (left) and section analyses (right). (C) Statistical analysis of DNA 7 and DNA 8 in a 2 x 2 um^2

area. (D) Left: AFM image of the DNA 9. The DNA sample used for this AFM examination was synthesized in the same way shown in Fig. S1. The sequence of DNA 9 was shown in Tab. 15. Right: Section analyses of one of an AFM image obtained from Fig. 8D. The presence of G-quadruplex structures was verifiable by the naked eyes (left) and section analyses (right). (E) Left: AFM image of the DNA 10. The DNA sample used for this AFM examination was were prepared in the same ways as for those loaded into Lane 1 to Lane 4 in Fig. 2D except that DNA 9 were not used as the substrate. Right: Section analyses of one of an AFM image obtained from Fig. 8E. The absence of G-quadruplex structures was verifiable by the naked eyes (left) and section analyses of one of an AFM image obtained from Fig. 8E. The absence of G-quadruplex structures was verifiable by the naked eyes (left) and section analyses (right). (F) Statistical analysis of DNA 9 and DNA 10 in a 2 x 2 um^2 area.

Name of DNA	Nucleotide sequence
Primer 1	5'CCGAGCTCAGGATCCGGATGATCCCCAAAACCCCAAAACC
	CCAAAACCCCAGTCCGTAATACGACTCAC 3'
Primer 2	5'TCGTTTGGTATGGCTTCATT 3'
Primer 3	5'GTGGATCCTCGTCGCAAAAC 3'
Primer 4	5'CCGGATCCATGGTTAACCCC 3'
Primer 5	5'CCGAGCTCCCGTAATACGACTCACTTA 3'
Primer 6	5'CGTCGTTTGGTATGGCTTCATTGAG 3'

Tab.	S1 .	Nucleo	otide s	equences	of	primers	used i	in our	poly	ymerase	chain	reactions.
				1		1						

Tab. S2. Nucleotide sequences of DNA S1.

5' CCGAGCTCAGGATCCGGATGATCCCCAAAACCCCAAAACCCCAGACCCGTAATACGACTCACTTAAGGCCTTGACTAGAGGGTAC 3' GGCTCGAGTCCTAGGCCTACTAGGGGTTTTGGGGTTTTGGGGTTTTGGGGTCAGGCATTATGCTGAGTGAATTCCGGAACTGATCTCCCATG

 ${\tt TTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTAATATCCAATTACAGTACTATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCCTTTACACGCGCCTTGGGGATAAACAAATAAAAA$

 $\label{eq:construct} a transformation of the second state of the$

Tab. S3. Nucleotide sequences of DNA S2.

5' caggatccggatgatccccaaaacccccaaaacccccagtccgtaatacgactcacttaaggccttgactagagggtac 3' tcgagtcctaggcctactaggggttttggggttttggggttttggggtcaggcattatgctgagtgaattccggaactgatctcccatg

 ${\tt TTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTATTTTTAATATCCAATTACCAATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAAGCCCCTTTACACGCGCCCTTGGGGATAAACAAATAAAAA$

CCTTGATCGAAGGAGAGAGAGAGCTGGAGCT 3' GGAACTAGCTTCCTCTCTCTCGACC 5'

Tab. S4. Nucleotide sequences of DNA S3.

CCTTGATCGAAGGAGAGAGAGAGCTG 3'>

Notes: (1) DNA S3 is a circular DNA; and

Tab. S5. Nucleotide sequences of DNA 1.

G-quadruplex region

 ${\tt TTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTATTTTTAATATCCAATTACAGTACTATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAAGCCCCTTTACACGCGCCCTTGGGGATAAACAAATAAAAA$

 $\substack{ \texttt{CCTTGATCGAAGGAGAGAGAGAGGCTG 3'} \\ \texttt{GGAACTAGCTTCCTCTCTCTCGAC 5'} }$

Notes: (1) DNA 1 is a circular DNA; and

Tab. S6. Nucleotide sequences of DNA S6.

CCTTGACTAGAGGGTACCAACCTAGGTATCTAGAACGAATTCCGGAGCCTGAATCGGCCAACGCGGGGAGAGGCGGTTTGCGTATTGGGC GGAACTGATCTCCCATGGTTGGATCCATAGATCTTGCTTAAGGCCTCGGACTTAGCCGGTTGCGCCCCCTCTCCGCCAAACGCATAACCCG ${\tt TCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAATCAAGGCCAGCAAAAGGCCAGGAACCGTAAACAAGGCCGCGTTGCTGGC$ AGGTGTCTTAGTCCCCTATTGCGTCCTTTCTTGTACACTCGTTAGTTCCGGTCGTTTTCCGGTCCTTGGCATTTGTTCCGGCGCAACGACCG GTGACGAGCATCACAAACAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCGACTAGTGCCCT CACTGCTCGTAGTGTTTGTTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCCTGATATTTCTATGGTCCGCAAAGGCTGATCACGGGA GATAATGATTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTAAATACATTCAAATATGTATCCGCTCTTTATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCCTTTACACGCGGCTTGGGGATAAACATTTATGTAAGTTTATACATAGGCGACTGAATGTATTTAGCGCCAGGGTTTTCCCAGTCACGACCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGGACTTACAAAAATCGCGGGTCCCAAAAAGGGTCAGTGCTGGCGTGTAAAAGGGGCTTTTCACGGGTGGACTGCAGATTCTTTGGTAATAATAGTAC $\label{eq:construct} a construct of the construction of the cons$ ATCCGG 3' TAGGCC 5'

Tab. S7. Nucleotide sequences of DNA S7.

5' CCTCGATGAAAGATCCTTTCCGGGAGATCCTTGATTCGAGCATAGCTGGCTG
CCTTGACTAGAGGGTACCAACCTAGGTATCTAGAACGAATTCCGGAGCCTGAATCGGCCAACGCGGGGGGGG
GCTCTTCCGCTTCCTCGCTCACTGATTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCA
TCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAATCAAGGCCAGCAAAAGGCCAGGAACCGTAAACAAGGCCGCGTTGCTGGC AGGTGTCTTAGTCCCCTATTGCGTCCTTTCTTGTACACTCGTTAGTTCCGGTCGTTTTCCGGTCCTTGGCATTTGTTCCGGCGCAACGACCG
eq:gagcatcacaaacaatcgacgctcaagtcagagtggcgaaacccgacaggactataaagataccaggcgtttccgactagtgccct cactgctcgtagtgtttgttagctgcgagttcagtctccaccgctttgggctgtcctgatatttctatggtccgcaaaggctgatcacggga
${\tt GGAAGCTCCCTCGTGCGCTCATAAGAAGGAGAGAAGCTAAGAGAGGAACTGGACTCTCAAACATGAAACGTTTTGTTATAGGTTAATGTCATCCTTCGAGGGAGCACCGCGAGTATTCTTCCTCTCTCT$
GATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTAAATACATTCAAATATGTATCCGCT CTATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCCCTTTACACGCGCCTTGGGGATAAACATTTATGTAAGTTTATACATAGGCGA
CATGATACAATAAGTCTCCCCTGATAAATGCTTCAATGAAGGAAG
AACATGGGGGGATCATGTAACTCGCCTTGATCGGAGCTGAATGAA
CTGAATGTATTTAGCGCCAGGGTTTTCCCAGTCACGACCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATG GACTTACATAAATCGCGGTCCCAAAAGGGTCAGTGCTGGCGTGTAAAGGGGGCTTTTCACGGTGGACTGCAGATTCTTTGGTAATAATAGTAC
actcctgtgtgaaattgttatccgctcacgaggccctttcgccgcgcgtttcggtgatgacggtgaaaacctctgacacatgcaggtcgaggcgcacacactttagcgagagtggtcgcggaaggcgcgcaaagccactactgccacttttggagactgtgtgcggaggg
GGAGGCGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGGCTGGC CCTCCGCCAGTGTCGAACAGACATTCGCCTACGGCCCTCGTCTGTTCGGGCAGTCCCGCGCAGTCGCCCACAACCGCCCACAGCCCGACCG
TTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGGACATATTGTCGTTACCGAATTCATGGACTAGTGAATCGTATTAC AATTGATACGCCGTAGTCTCGTCTAACATGACTCTCACGTGGTATAACCGGTATAACAGCAATGGCTTAAGTACCTGATCACTTAGCATAATG
GTCTGTGTGATTGTTATCCGAGCTTATCAAACCACCGCCCGC

Tab. S8. Nucleotide sequences of DNA S8.

 $\overline{<_{3'iactgatctccatggttggatccataggtttgcatcttagatcttagacgaattccggacctgaatcggccgatggcgggagaggcggtttgcgtattgggccgataacgcataacgcctggacttagccggttgcgcccctctcccgccaaacgcataacccg}}$ $\label{eq:gcacctcctcgtgcgctcataagaaggaggaggaggaggaggaggagcaccggacctccatgaaacgtttgttataggttaatgtcatgtcctccagggaggagcacgcgaggattcttcctcctctcgagaggttgtactttgcaaaacaatatccaattacagta$ GATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTAAATACATTCAAATATGTATCCGCTCTATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCCTTTACACGCGGCCTTGGGGATAAACATTTATGTAAGTTTATACATAGGCGACTGAATGTATTTAGCGCCAGGGTTTTCCCCAGTCACGACCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATG ACTCCTGTGTGAAATTGTTATCCGCTCACGAGGCCCTTTCGCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCC TGAGGACACACTTTAACAATAGGCGAGTGCTCCGGGAAAGCGGAGCGCGCAAAGCCACTACTGCCACTTTTGGAGACTGTGTACGTCGAGGG GTCTGTGTGTGATTGTTATCCGAGCTTATCAAACCACCGCTCGCCAAAAGGATCTCCGGGAAAGGATCTTTCATCGAGCTCCTCGATGAAAGATC CTTTCCGGAGATCCTTGATTCGAGCATAGCTGGCTGGTGTTGCGGCAGTCCGCC 3'

Notes: (1) DNA S8 is a circular DNA; and

Tab. S9. Nucleotide sequences of DNA 3.

5'AACCTAGGTATCTAGAACGAATTCCGGAGCCTGAATCGGCCAACGCGGGGGGAGAGGCGGTTTGCGTATTGGGCGCCTCTTCCGCTTCCT' 3'TTGGATCCATAGATCTTGCTTAAGGCCTCGGACTTAGCCGGTTGCGCGCCCCTCTCCGCCAAACGCATAACCCGCGAGAAGGCGAAGGA
GCTCACTGATTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCA
ATAACGCAGGAAAGAACATGTGAGCAATCAAGGCCAGCAAAAGGCCAGGAACCGTAAACAAGGCCGCGTTGCTGGCGTGACGAGCATCACA TATTGCGTCCTTTCTTGTACACTCGTTAGTTCCGGTCGTTTTCCGGTCCTTGGCATTGTTCCGGCGCAACGACCGCACTGCTCGTAGTGT
ACAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCGACTAGTGCCCTGGAAGCTCCCTCGTG TGTTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCCTGATATTTCTATGGTCCGCAAAGGCTGATCACGGGACCTTCGAGGGAGCAC
GCTCATAAGAAGGAGGAGGAAGCTAAGAGAGGAACTGGACTCTCAAACATGAAACGTTTTGTTATAGGTTAATGTCATGATAATAATGGTTTC CGAGTATTCTTCCTCTCTCTCGATTCTCCCTTGACCTGAGAGTTTGTACTTTGCAAAACAATATCCAATTACAGTACTATTATTACCAAAG
TAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTAAATACATTCAAATATGTATCCGCTCATGATACAATAAGT ATCTGCAGTCCACCGTGAAAAGCCCCTTTACACGCGCCTTGGGGATAAACATTTATGTAAGTTTATACATAGGCGAGTACTATGTTATTCA
TCCCCTGATAAATGCTTCAATGAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTGCACAACATGGGGGGATCAT AGGGGACTATTTACGAAGTTACTTCCTTCTTCATACTCATAAGTTGTAAAGGCACAGCGGGAATAAGGGAAAACGTGTTGTACCCCCTAGTA
TAACTCGCCTTGATCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCTCGAGCCCTGAATGTATTTAGC ATTGAGCGGAACTAGCCTCGACTTACTTCGGTATGGTTTGCTGCTCGCACTGTGGTGCTACGGACGTCGAGCTCGGGACTTACATAAATCG
CCAGGGTTTTCCCAGTCACGACCGCACATTTCCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACTCCTGTGTGAAAT GGTCCCAAAAGGGTCAGTGCTGGCGTGTAAAGGGGGCTTTTCACGGTGGACTGCAGATTCTTTGGTAATAATAGTACTGAGGACACACTTTA
GTTATCCGCTCACGAGGCCCTTTCGCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGGCGGTCACAGC CAATAGGCGAGTGCTCCGGGAAAGCGGAGCGCGCAAAGCCACTACTGCCACTTTTGGAGACTGTGTACGTCGAGGGCCTCCGCCAGTGTCG
TGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGGTGTCGGGGCTGGCT
AGAGCAGATTGTACTGAGAGTGCACCATATGGACATATTGTCGTTACCGAATTCATGGACTAGTGAATCGTATTACGTCTGTGTGTG
crucif orm region
TCCGAGCTTATCAAACCACCGCTCGCCAA AAGGATCTCCGGAAAGGATCTTTCATCGAGCTCCTCGATGAAAGATCCTTTCCGGAGAATCCT AGCCTCGAATAGTTTGGTGGCGAGCGGTT
TTCCTAGAGGCCTTTCCTAGAAAGTAGCTCGAGGAGCTACTTTCTAGGAAAGGCCTCTAGGA.
cruciform region
GATTCGAGCATAGCTGGCTGGTGTTGCGGCAGTCCGCCTTGACTAGAGGGTACC 3'
CTAAGCTCGTATCGACCGACCACAACGCCGTCAGGCGGAACTGATCTCCCATGG 5'

Notes: (1) DNA 2 is a circular DNA; and

Tab. S10. Nucleotide sequences of DNA S10.

5' CCGAGCTCCCGTAATACGACTCACTTAAGGCCTTGACTAGAGGGTACCAACCTAGGTATCTAGAACCGGTCTCGAGCCATAACTTCGTATAG 3' GGCTCGAGGGCATTATGCTGAGTGAATTCCGGAACTGATCTCCCATGGTTGGATCCATAGATCTTGGCCAGAGCTCGGTATTGAAGCATATC

 ${\tt GGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGCCGTGAAAAAGCCCCTTTACACGCGCCCTTGGGGATAAACAAATAAAAAGATTTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGAC$

 $\label{eq:construct} A GCGGTA A GTTA A GCTTTTTGCA CAACATGGGGGGATCA TGTA A CTCGCCTTGA TCGA A GGA GGA GCTGGA GCTCA A TGA A GCCCTTGA A CTCGCCTTGA A CTCGCCTTGA A CTCGCCTTGA A CTCGCCTTGA A CCCCCTA GTA A CTTGA GCGGA A CTA GCTTCCTCTCTCTCGA CTCGA GTA A CTTCGGTA TGG A CTA GCTTCGA A CTTCGA A CTTGA A CTTCGA A CTTCGA$

AAACGACG 3' TTTGCTGC 5'

Tab. S11. Nucleotide sequences of DNA S11.

Tab. S12. Nucleotide sequences of DNA S12.

<5. GAGCTCCCGTAATACGACTCACTTAAGGCCTTGACTAGAGGGTACCAACCTAGGTATCTAGAACCGGTCTCGAGCCATAACTTCGTATAG 3. CTCGAGGGCATTATGCTGAGTGAATTCCGGAACTGATCTCCCATGGTTGGATCCATAGATCTTGGCCAGAGCTCGGTATTGAAGCATATC

 $\label{eq:construct} a transformed a construct construction of the second sec$

Notes: (1) DNA S12 is a circular DNA; and

Tab. S13. Nucleotide sequences of DNA 5.



Notes: (1) DNA 5 is a circular DNA; and

Tab. S14. Nucleotide sequences of DNA 7.

Notes: (1) DNA 7 is a circular DNA; and

Tab. S15. Nucleotide sequences of DNA 9.

5' GAGCTCAGGATCCGGATGAT CCCCCAACCCCAACCCCAACCCC AGTCCGTAATACGACTCACTTAAGGCCTTGACTAGAGGGGTAC CTCGAGTCCTAGGCCTACTA GGGGTTGGGGTTGGGGTTGGGGTTGGGGTTGGGGATTATGCTGAGTGAATTCCGGAACTGATCTCCCATG I
G-quadruplex region
${\tt caacctaggtatctagaaccggtctcgagccataacttcgtatagcatacattatacgaagttatatagcatgtcgaactgtcaaacatgagaattcttggttgg$
${\tt TTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTAATATCCAATTACAGTACTATTATTACCAAAGAATCTGCAGTCCACCGTGAAAAGCCCCTTTACACGCGCCTTGGGGGATAAACAAATAAAAAAAA$
CTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACGAATTATGTAAGTTTATACATAGGCGAGTACTCTGTTATTGGGACTATTTACGAAGTTATTATAACTTTTTCCTTCTCATACTCATAAGTTG
$\begin{tabular}{lllllllllllllllllllllllllllllllllll$
${\tt GATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGTTAAGCTTTTTGCACAACATGGGGGATCATGTAACTCGCTAGTCAACCCACGTGCTCACCCCAATGTAGCTTGACCTAGAGTTGTCGCCATTCAATCCGAAAAACGTGTTGTACCCCCTAGTACATTGAGCCTAGTCAACTCGAAAAACGTGTTGTACCCCCTAGTACATTGAGCCTAGTCAACTCGAAAAACGTGTTGTACCCCCTAGTACATTGAGCCTAGTCAACTGACATGTAGCCTAGAGTTGTCGCCATTCAATTCGAAAAACGTGTTGTACCCCCTAGTACATTGAGCCTAGTCAACTGAGCTTGTCGCCATTCAATTCGAAAAACGTGTTGTACCCCCTAGTACATTGAGCCTAGTGTCGCCATTCAATTCGAAAAACGTGTTGTACCCCCTAGTACATTGAGCCTAGTGTCGCCATTCAATTCGAAAAACGTGTTGTACCCCCCTAGTACATTGAGCCTAGTGTCGCCATTCAATTCGAAAAACGTGTTGTACCCCCTAGTACATTGAGCCTAGTAGTTGTCGCCATTCAATTCGAAAAACGTGTTGTACCCCCTAGTACATTGAGCCTAGTAGTTGTACCCCCTAGTACATTGAGCCTAGTACATTGAGCTTGTACCCCCTAGTACATTGAGCCTAGTGTGTGT$
CCTTGATCGAAGGAGAAGAGCTG 3'>

Notes: (1) DNA 9 is a circular DNA; and

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