# Electronic Supplementary Information

## Design and synthesis of catenated rings based on toroidal DNA structures

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## Materials and Methods:

**Reagents:** *Taq* DNA Polymerase, T4 DNA ligase, Nuclease BAL-31, Sac I and Cre recombinase were purchased from New England Biolabs (Ipswich, MA). Human Topoisomerase I was obtained from TopoGEN (Columbus, OH). pGH plasmid that contains our newly designed DNA sequence was provided by Generay Biotech (Shanghai, China) and single strand oligonucleotides were produced by Sigma-Aldrich (Singapore).

**PCR amplification of Linear DNA 1:** A reported standard PCR amplification process[1-2] was followed in our studies to obtain Linear DNA 1 using oligonucleotides 5'GTGGATCCTCGTCGCAAAAC3' and 5'CCGGATCCATGGTTAACCC3' as forward and reverse primers and pGH plasmid as template.

**Preparations of Linear DNA 2:** A mixture containing 2 pmol Linear DNA 1, 5 U of Sac I, 10 mM Bis-Tris-Propane-HCl, 10 mM $MgCl_2$, 1 mM Dithiothreitol was incubated 37 ℃ for 2 hours. The resultant cohesive end-containing Linear DNA 2 was purified using the QIAquick Purification Kits (Qiagen).

**Preparations of Circular DNA 1:** A mixture containing 2 pmol Linear DNA 2, 60 mM Tris–HCl (pH 7.6), 25 mM NaCl, 13 mM $MgCl_2$, 10 mM DTT, 1mM ATP, 25 mg/ml BSA and 10 U of T4 DNA ligase was incubated at 16 ℃ for 16 hours. The resultant mixture was further incubated with BAL-31 at 30 ℃ for 2 hours, which were then analyzed using 2.5% agarose gel electrophoresis. The DNA product that is corresponding to Band 1 in Lane 5 of Figure 2B was purified next using Mini Prep Cell (Bio-rad) before AFM examination.

**Reaction of relaxed form of Circular DNA 1 with Human Topoisomerase I:** A mixture containing 0.2 pmol circular DNA 1, 1 U of Human Topoisomerase I, 10 mM Tris–HCl (pH 7.9), 150 mM NaCl, 0.1% bovine serum albumin (BSA), 0.1 mM spermidine and 5% glycerol was incubated at 37 ℃ for 1 hour followed by analysis using 2% agarose gel electrophoresis.

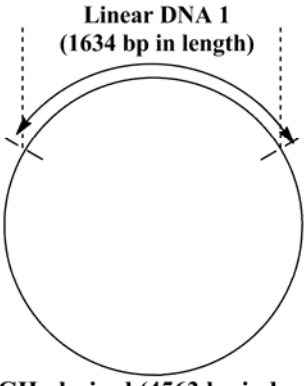
**Formation of catenated DNA:** A mixture containing 0.2 pmol Circular DNA 1, 50 mM Tris-HCl, 33 mM NaCl, 10 mM $MgCl_2$ and 1 U of Cre recombinase was incubated at 37 ℃ for 12 hours. The resultant reaction mixture was further analyzed using 2.5% agarose gel electrophoresis.

**AFM studies of our synthesized catenated DNA**

*(a) Immobilization of DNA samples on micas*[3-5]*:* A buffer (PH 7.0) containing 40 mM HEPES and 10 mM $MgCl_2$ was placed on freshly cleaved mica, which was further kept at room temperature for 5 min. The pre-prepared mica was rinsed with dd water thoroughly and dried under the argon flow. A 20 μL solution that contains DNA sample was deposited on the mica, which was subsequently incubated for 5 min, washed with $H_2O$, dried under the argon flow and then stored in vacuum

cabinet.

*(b) AFM examination*: AFM images of DNA were obtained in Tapping Mode™ on a Multimode™ AFM (Veeco, Santa Barbara, CA) in connection with a Nanoscope V™ 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 [6-7]. All DNA sample determinations were carried out in air at room temperature.




## Nucleotide sequence of Linear DNA 1 (PCR product from pGH plasimd)

5'GTGGATCCTCGTCGCAAAACGAGCTCAGTTGGGTAATTTTTAGGGTTTTCCCAGTTTTGACGTTGTTTTTCGACGGAATTCCCTTTTTACGACTCACTTTTTGCCTTGACTAGA
3'CACCTAGGAGCAGCGTTTTGCTCGAGTCAACCCATTAAAAATCCCAAAAGGGTCAAAACTGCAACAAAAAGCTGCCTTAAGGGAAAAATGCTGAGTGAAAAACGGAACTGATCT

GGGTTTTTACCCCATGGTCTATTTTTGGTCTTTTGCCATAACTTTTTATAGCATACATTTTACGAGTTTTATAAGCTGTTTTTGCCATAACTTTTTGCCATAACTTTTTATAGCAT
CCCAAAAATGGGGTACCAGATAAAAACCAGAAAACGGTATTGAAAAATATCGTATGTAAAATGCTCAAAATATTCGACAAAAACGGTATTGAAAAACGGTATTGAAAAATATCGTA

ACATTTTACGAGTTTTATAAGCTGTTTTTGCCATAACTTTTTATAGCATACATTTTACGAGTTTTATAAGCTGTTTTTCATGAGGCTTCTTTTTTATAGGTTTTTGTCATGATTTTA
TGTAAAATGCTCAAAATATTCGACAAAAACGGTATTGAAAAATATCGTATGTAAAATGCTCAAAATATTCGACAAAAAGTACTCCGAAGAAAAATATCCAAAAACAGTACTAAAAT

ATGGTATCTTTTTGCCATAACTTTTTATAGCATACATTTTACGAGTTTTATAAGCTGTTTTTCATGAGGCTTCTTTTTTATAGGTTTTTGTCATGATTTTAATGGTATCTTTTTCGT
TACCATAGAAAAACGGTATTGAAAAATATCGTATGTAAAATGCTCAAAATATTCGACAAAAAGTACTCCGAAGAAAAATATCCAAAAACAGTACTAAAATTACCATAGAAAAAGCA

CGGTGGCATTTTTCGGGGTTTTGCGCGGATGCCTTTTTGTTTATGGGCCTTTTTACATCAGGTTTTTTCCGCTCAGCAATGATTTTTGCCCTTTTAGATTTTTCAATGATATTTTT
GCCACCGTAAAAAGCCCCAAAACGCGCCTACGGAAAAACAAATACCCGGAAAAATGTAGTCCAAAAAAGGCGAGTCGTTACTAAAAACGGGAAAATCTAAAAAGTTACTATAAAAA

AGGCGTTTTTGACGTTTTCAGTTTTTCCGTGTCGCCCTTTTTCCCTTTTTTGCGCATTTTTTCGGCACTTTTTTGCATATATTTTTTGGAGTTGTTTTGATCCGTTTTGATTTTCA
TCCGCAAAAACTGCAAAAGTCAAAAAGGCACAGCGGGAAAAAGGGAAAAAACGCGTAAAAAAGCCGTGAAAAAACGTATATAAAAAACCTCAACAAAACTAGGCAAAACTAAAAGT

Lox P site 1

GTGCGTTTTTGGCCATTTTGCCTAGTTTTTTATAACTTCGTATAGCATACATTATACGAAGTTATTTGCTATTTTTTTGTTAATTTTTGCCAATTTTCGTATTTTCGCTATTTTTTG
CACGCAAAAACCGGTAAAACGGATCAAAAAATATTGAAGCATATCGTATGTAATATGCTTCAATAAACGATAAAAAACAATTAAAAACGGTTAAAAGCATAAAAGCGATAAAAAAC

GCATTTTTTGACCATTTTTCTTGTTTTGCCAAGTTTTCGGCCGTTTTTTGGAGTTGAATTTTACGTCCAGATTTGATTTTCAGTGCGTTTTTAAGCTTTTTTGCAGTGTTTTTTGC
CGTAAAAAACTGGTAAAAAGAACAAAACGGTTCAAAAGCCGGCAAAAAACCTCAACTTAAAATGCAGGTCTAAACTAAAAGTCACGCAAAAATTCGAAAAAACGTCACAAAAAACG

CTCTGCTATTTTTTGTTAATTTTTGCCAATTTTCGAGGTATTTTCGCTATTTTTTGGCATTTTTTGCTGCATTTTTTAGTCGTTTTTGGCCATTTTTCCTGTTTTTGCTCACCCAT
GAGACGATAAAAAACAATTAAAAACGGTTAAAAGCTCCATAAAAGCGATAAAAAACCGTAAAAAACGACGTAAAAAATCAGCAAAAACCGGTAAAAAGGACAAAAACGAGTGGGTA

TTTCGCTGGTGCCGAGTTTTTGATGCTTTTTGCAGTTTTGTGCACGAGTTTTTGACATCGGACTGGTTTTCACAGCGGTTTTCAGGCTTTTTGCACAACATTTTTAAGCTTTTTTG
AAAGCGACCACGGCTCAAAAACTACGAAAAACGTCAAAACACGTGCTCAAAAACTGTAGCCTGACCAAAAGTGTCGCCAAAAGTCCGAAAAACGTGTTGTAAAAATTCGAAAAAAC

AAGGAGAGAAGATTTTGGGTCTCAGTTTTGATACCCGACGATTTTGACACCACGATTTTTGCAGGCGTTTTGGGCTCAGTTTTGATACCCGACGATTTTGACACCACGATTTTTGC
TTCCTCTCTTCTAAAACCCAGAGTCAAAACTATGGGCTGCTAAAACTGTGGTGCTAAAAACGTCCGCAAAACCCGAGTCAAAACTATGGGCTGCTAAAACTGTGGTGCTAAAAACG

Lox P site 2

AGGCGTTTTTGAGCCATTTTTGCGTATAGCATTTTGGATACGATGTTTTCCATGATAACTTCGTATAGCATACATTATACGAAGTTATTTTTGGGCTCAGTTTTGATACCCGGATC
TCCGCAAAAACTCGGTAAAAACGCATATCGTAAAACCTATGCTACAAAAGGTACTATTGAAGCATATCGTATGTAATATGCTTCAATAAAAACCCGAGTCAAAACTATGGGCCTAG

TTTTGACACCACGATTTTTGCAGGCGTTTTTGAGCCATTTTTGCGTATAGCATTTTGGATACGATGTTTTCCATGTTTTAGTGGTTTTTGACGTAGCTTTTCGCCATGGTAGTCAT
AAAACTGTGGTGCTAAAAACGTCCGCAAAAACTCGGTAAAAACGCATATCGTAAAACCTATGCTACAAAAGGTACAAAATCACCAAAAACTGCATCGAAAAGCGGTACCATCAGTA

TTTTATAGCTGTTTTTGTGTGAGATTTTTATCCGCTCACTTTTCGAATTCCTTTTTACGAGCCGGATTTTTGCGGTGTGGCTTTTTGTCCGTTTTCCTGTTTGAGCTCGGGGTTAA
AAAATATCGACAAAAACACACTCTAAAAATAGGCGAGTGAAAAGCTTAAGGAAAAATGCTCGGCCTAAAAACGCCACACCGAAAAACAGGCAAAAGGACAAACTCGAGCCCCAATT

CCATGGATCCGG 3'
GGTACCTAGGCC 5'

Figure S1

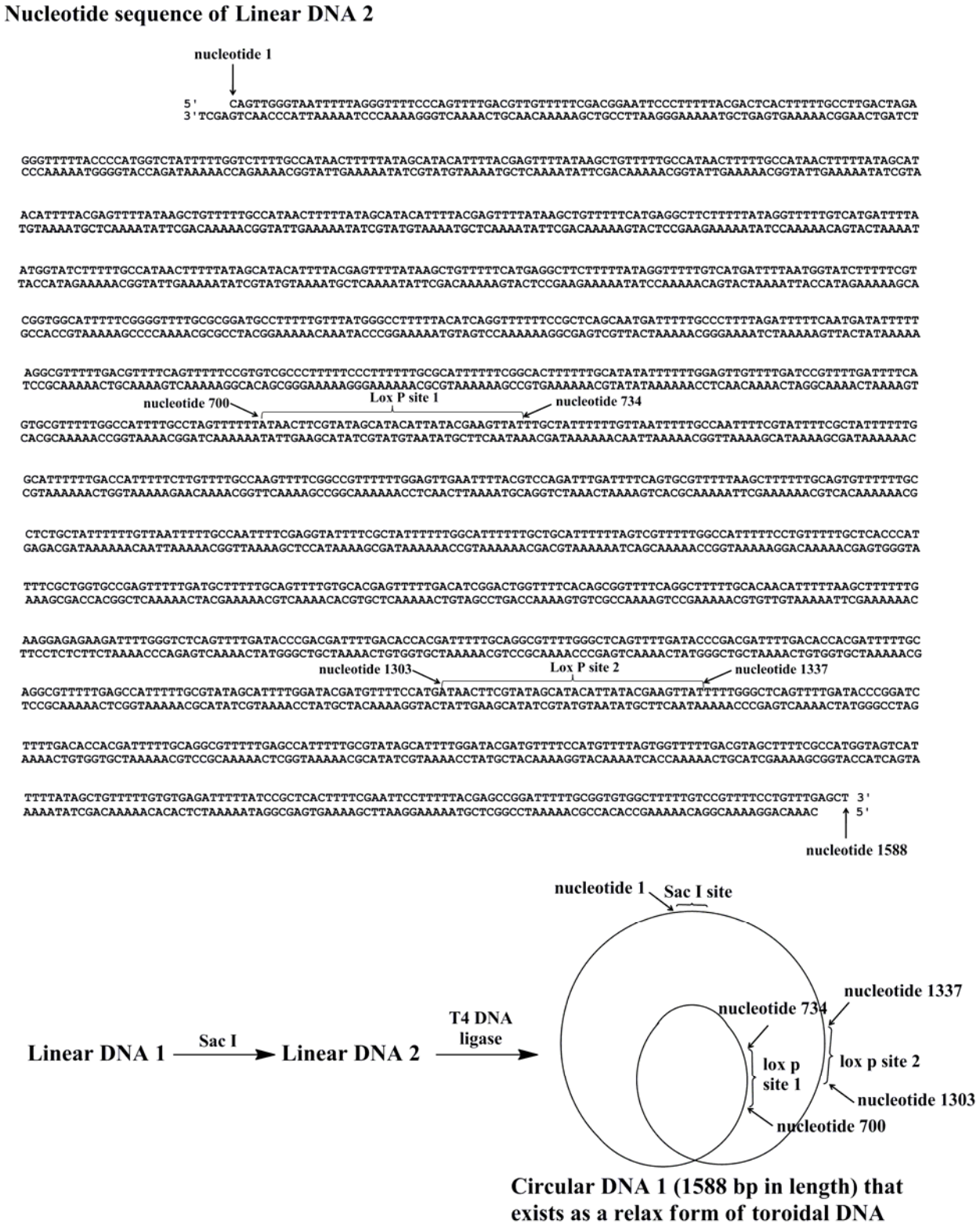

Nucleotide sequence of Linear DNA 2
nucleotide 1
5' CAGTTGGGTAATTTTTAGGGTTTTCCCAGTTTTGACGTTGTTTTTCGACGGAATTCCCTTTTTACGACTCACTTTTTGCCTTGACTAGA
3' TCGAGTCAACCCATTAAAAATCCCAAAAGGGTCAAAACTGCAACAAAAAGCTGCCTTAAGGGAAAAATGCTGAGTGAAAAACGGAACTGATCT
nucleotide 700
Lox P site 1
nucleotide 734
nucleotide 1303
Lox P site 2
nucleotide 1337
nucleotide 1588
nucleotide 1
Sac I site
Linear DNA 1
Sac I
Linear DNA 2
T4 DNA ligase
nucleotide 1337
nucleotide 734
lox p site 1
lox p site 2
nucleotide 1303
nucleotide 700
Circular DNA 1 (1588 bp in length) that exists as a relax form of toroidal DNA

Figure S2

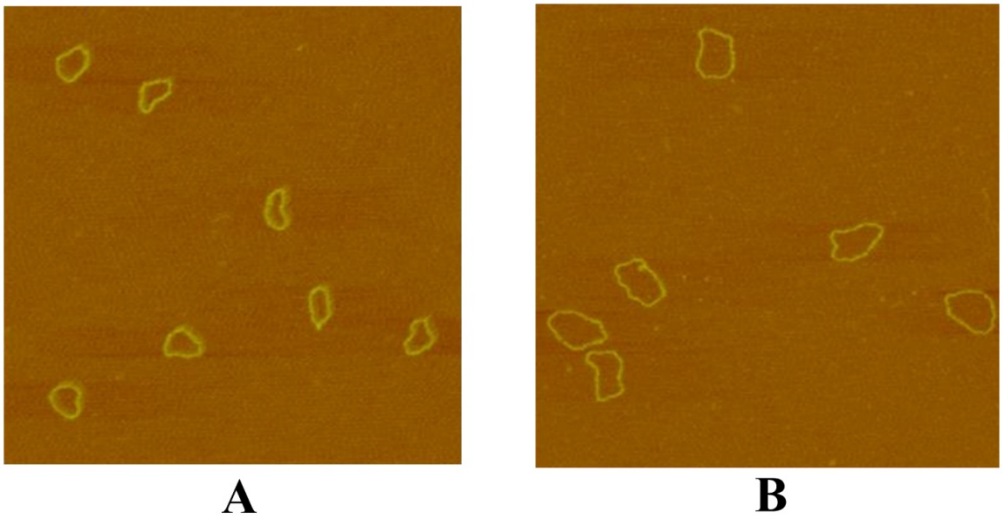



Figure S3. AFM images of Non-Catenated Ring 1 and Non-Catenated Ring 2. A: AFM image of Non-Catenated Ring 1 (603 bp in length) that are corresponding to Band 4 in Lane 3 in Figure 3A (1 μm x 1 μm scans). B: AFM image of Non-Catenated Ring 2 (985 bp in length) that are corresponding to Band 3 in Lane 3 in Figure 3A (1 μm x 1 μm scans).

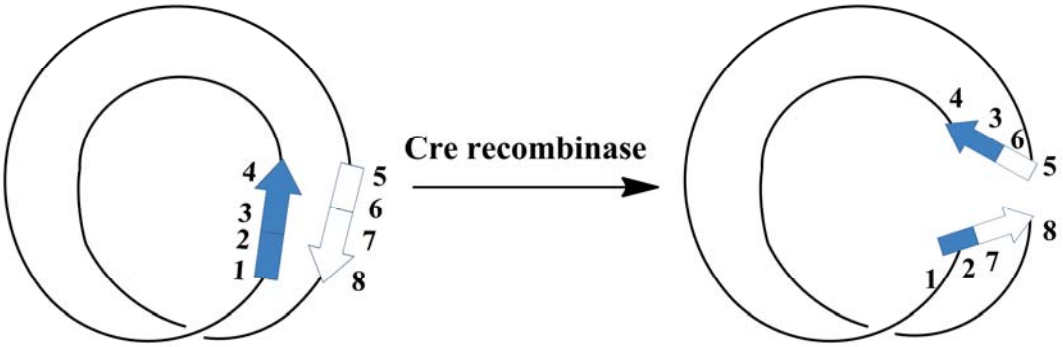



Figure S4. Schematic illustration of strand recombination of toroidal DNA in which two lox p sites are aligned in an anti-parallel fashion. This Cre recombinase-catalyzed reaction does not lead to any catenated DNA structure. ( indicates the lox p site)

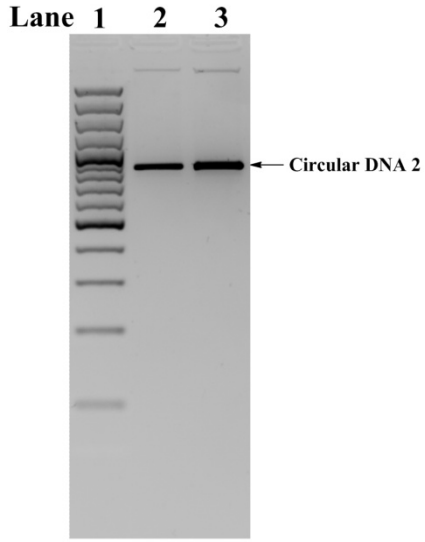



Figure S5. Electrophoretic analysis of reactions between Circular DNA 2 and Cre recombinase. Lnae 1: molecular weight markers; Lane 2: Circular DNA 2 alone; Lane 3: reaction mixture of Circular DNA 2 and Cre recombinase.

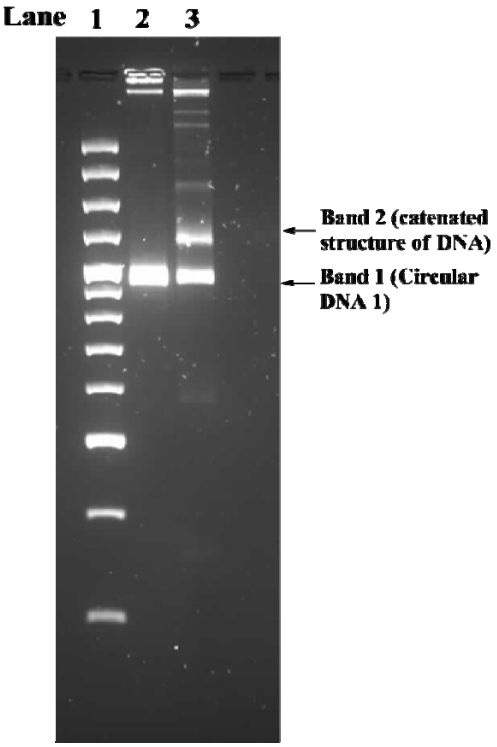



Figure S6. Optimization of reaction yield for formation of catenated DNA structure. Land 1: molecular weight markers; Lane 2: Circular DNA 1 alone; Lane 3: reaction mixture of Circular DNA 1 and Cre recombinase. The reaction mixture loaded in Lane 3 was prepared as follows : 4 U (1 U each time) of Cre recombinase was added to a solution containing 0.2 pmol Circular DNA 1, 50 mM Tris-HCl, 33 mM NaCl and 10 mM $MgCl_2$ for four times within 20 minute intervals. After each addition of Cre recombinase, the resultant solution was further incubated at 37 ℃. The reaction yield is ～16%.

## References for Supplementary Information: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5. F. Moreno-Herrero, L. Holtzer, D. A. Koster, S. Shuman, C. Dekker and N. H. Dekker, *Nucleic Acids Research*, 2005, **33**, 5945-5953.
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