Electronic Supplementary Information

Small DNA circles as bacterial topoisomerase I inhibitors

Dawei Li, *[a,c] Qiang wang, [a] Bing Zhou, [a] Qiang Zhuge [a,c] and Bei Lv*[b]

a. College of biology and the environment, Nanjing Forestry University, 159 Longpan Road, Nanjing, 210037, China.

Email: dwli@njfu.edu.cn

b. The Southern Modern Forestry Collaborative Innovation Center, Nanjing Forestry University, 159 Longpan Road, Nanjing, 210037, China.

c. Jiangsu Key Laboratory for Biofunctional Molecules, College of Life Science and Chemistry, Jiangsu Second Normal University, Nanjing, 210013, China.

* Corresponding authors. Tel.: +86-8542-8701; fax: +86-8542-8701; e-mail: dwli@njfu.edu.cn, lida0006@e.ntu.edu.sg (D. Li)

Materials and Methods:

Reagents: Bacterial topoisomerase I (*Escherichia coli* Topoisomerase I) and T4 DNA ligase were obtained from New England Biolabs (Ipswich, MA). Plasmid pBR322, Exonuclease I and Exonuclease III were purchased from Takara Bio Inc. (Dalian, China). All the oligonucleotides were provided by Sangon Biotech (Shanghai, China) with HPLC purification, which were used as precursors for small DNA synthesis. The sequences of oligonucleotides were given in Tab. S1. All the buffer and solution are prepared by the biological purity water.

Synthesis of small circular DNAs: All the DNA circles were prepared using Ligase Assisted Minicircle Accumulation (LAMA) according to previous literature report[1]. Generally, a reaction mixture containing oligonucleotides and reaction buffer (60 mM Tris–HCl (pH 7.6), 25 mM NaCl, 13 mM $MgCl_2$,10 mM DTT, 1mM ATP, 25 mg/ml BSA) was heated to 95℃ for 5 min and then chilled immediately on ice for 5min. The obtained mixture was brought to 16℃ and incubated with 10 U of T4 DNA ligase overnight. After that, the DNA products were treated by Exonuclease I and Exonuclease III to digest the remaining single and double-stranded linear DNA. The minicircles were purified subsequently by the PCR Purification Kit or the Nucleotide Removal Kit (Qiagen). The minicircles did not contain chemically synthesized oligonucleotides. The obtained DNA circles were subsequently analyzed and purified by PAGE gel.

Reactions of bacterial topoisomerase I with supercoiled pBR322 plasmid: A 50 μl solution containing 50 mM Potassium Acetate, 20 mM Tris-acetate (pH = 7.9), 10

mM Magnesium Acetate, 100 μg/ml BSA, 100 ng pBR322 plasmid and 0.5 U (8.22 nM) *Escherichia coli* topoisomerase I was incubated with or without cODNs at 37 ℃ for 0.5 hr. The obtained products were further analyzed using agarose electrophoresis (1.0%) in the absence of ethidium bromide. The gel was photographed and the DNA bands were measured and quantified using Gel Documentation System (BioRad ChemiDocXRS, US).

Relaxation inhibition assay: 0.5 unit (8.22 nM) of *Escherichia coli* topoisomerase I was pre-incubated with a solution containing cODNs and reaction buffers at room temperature for 3 minutes. After that, 100 ng supercoiled pBR322 plasmid was added into above mentioned solution. The final reaction mixture was further incubated at 37 ℃ for 30 minutes. The obtained reaction mixture were analyzed as described above. Percentage of relaxation was defined as the ratio of band density of relaxed DNA over those of relaxed DNA plus supercoiled DNA: relaxed DNA/(relaxed DNA + supercoiled DNA)[2].

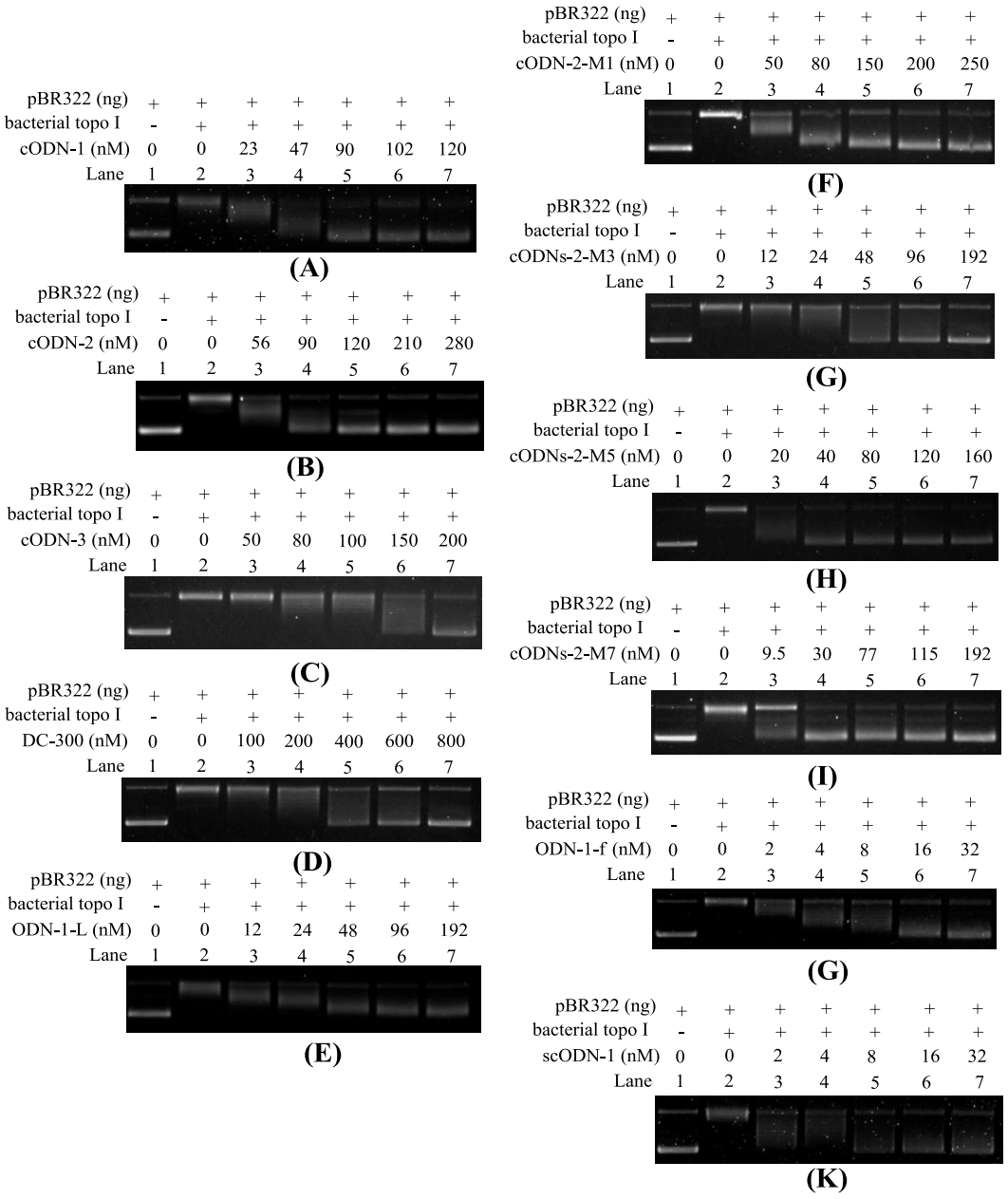

Fig. S1 Electrophoretic analysis of pBR322 relaxation catalyzed by bacterial topoisomerases I in the presence of cODN-1 (A), cODN-2 (B), cODN-3 (C), DC-300 (D), ODN-1-L (E), cODN-2-M1 (F), cODN-2-M3 (G), cODN-2-M5 (H), cODN-2-M7 (I), ODN-f-1 (J) or scODN-1 (K) as the inhibitors. The reaction mixtures containing 50 mM KAc, 20 mM Tris-Ac (pH 8.0), 10 mM $Mg(Ac)_2$, 100 mg/ml BSA, 100 ng pBR 322, 0.5 U (8.22 nM) of bacterial topoisomerases I and oligonucleotides were incubated at 37 ℃ for 30 min before loading on agarose gel.

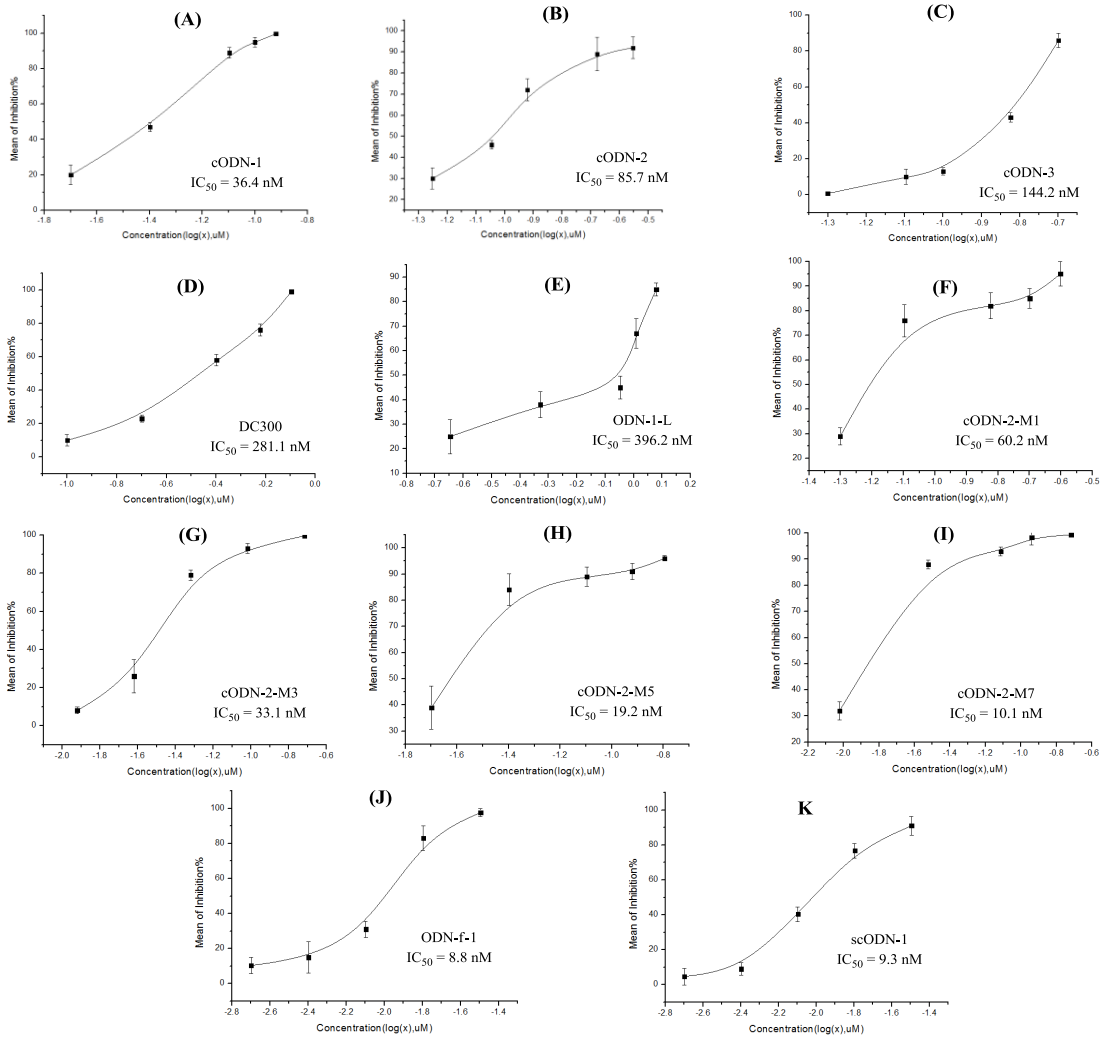

Fig. S2 Correlations between concentration of cODN-1 (A), cODN-2 (B), cODN-3 (C), DC-300 (D), ODN-1-L (E), cODN-2-M1 (F), cODN-2-M3 (G), cODN-2-M5 (H), cODN-2-M7 (I), ODN-1-f (J), scODN-1 (K) and the corresponding inhibitory effects on bacterial topoisomerases I. Percentage of relaxation was defined as the ratio of band density of relaxed DNA over those of relaxed DNA plus supercoiled DNA: relaxed DNA/(relaxed DNA + supercoiled DNA) in Fig S1. The gel was photographed and the DNA bands were measured and quantified using Gel Documentation System (BioRad ChemiDocXRS, US).

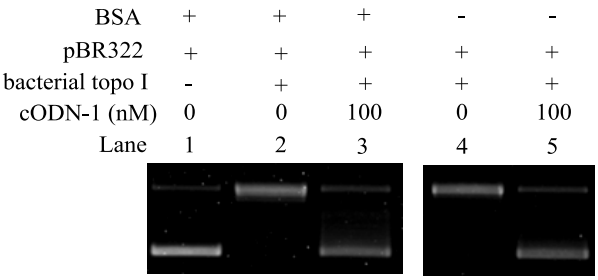

Fig. S3 The inhibition of pBR322 relaxation induced by cODN-1 in the reaction buffer with or without bovine serum albumin (BSA).

Tab. S1 The sequences of oligonucleotides used in this studied.

Oligonucleotide	Sequence
ODN-1-f	5'ATCAAGACCCGTGCAATGCTATCGACATCAAGGCCTAGTCAATGGGTTCAGGATGCAGGTGAGGAT3'
ODN-1-r	5'CCTTGATGTCGATAGCATTGCACGGGTCTTGATATCCTCACCTGCATCCTGAACCCATTGACTAGG3'
ODN-1-L	5'ATCCTCACCTGCATCCTGAACCCATTGACTAGGCCTTGATGTCGATAGCATTGCACGGGTCTTGAT3'
ODN-2-f	5'ATCTTCATCGAACAAGACCCGTGCAATGCTATCGACATCAAGGCCTATCGCTTGGGAGTCAATGGGTTCAGGATGCAGGTGAGGAT3'
ODN-2-r	5'CCTTGATGTCGATAGCATTGCACGGGTCTTGTTCGATGAAGATATCCTCACCTGCATCCTGAACCCATTGACTCCCAAGCGATAGG3'
ODN-3-f	5'ATCTTTGCGGCAGTTAATCGAACAAGACCCGTGCAATGCTATCGACATCAAGGCCTATCGCTATTACGGGGTTGGGAGTCAATGGGTTCAGGATGCAGGTGAGGAT3'
ODN-3-r	5'CCTTGATGTCGATAGCATTGCACGGGTCTTGTTCGATTAACTGCCGCAAAGATATCCTCACCTGCATCCTGAACCCATTGACTCCCAACCCCGTAATAGCGATAGG3'
ODN-2-r-M1	5'CCTTGATGTCGATAGCAGTGCACGGGTCTTGTTCGATGAAGATATCCTCACCTGCATCCTGAACCCATTGACTCCCAAGCGATAGG3'
ODN-2-r-M3	5'CCTTGATGTCGATAGCCGGGCACGGGTCTTGTTCGATGAAGATATCCTCACCTGCATCCTGAACCCATTGACTCCCAAGCGATAGG3'
ODN-2-r-M5	5'CCTTGATGTCGATAGCCGGCGACGGGTCTTGTTCGATGAAGATATCCTCACCTGCATCCTGAACCCATTGACTCCCAAGCGATAGG3'
ODN-2-r-M7	5'CCTTGATGTCGATAGCCGGCGGGGGGTCTTGTTCGATGAAGATATCCTCACCTGCATCCTGAACCCATTGACTCCCAAGCGATAGG3'

cODN-1 was produced with ODN-1-f and ODN-1-r by Ligase Assisted Minicircle Accumulation (LAMA).
ODN-1-L was produced with ODN-1-f and ODN-1-L by standard annealing.
cODN-2 was produced with ODN-2-f and ODN-2-r Ligase Assisted Minicircle Accumulation (LAMA).
cODN-3 was produced with ODN-3-f and ODN-3-r by Ligase Assisted Minicircle Accumulation (LAMA).
cODN-2-M1 was produced with ODN-2-f and ODN-2-M1 by Ligase Assisted Minicircle Accumulation (LAMA).
cODN-2-M3 was produced with ODN-2-f and ODN-2-M3 by Ligase Assisted Minicircle Accumulation (LAMA).
cODN-2-M5 was produced with ODN-2-f and ODN-2-M5 by Ligase Assisted Minicircle Accumulation (LAMA).
cODN-2-M7 was produced with ODN-2-f and ODN-2-M7 by Ligase Assisted Minicircle Accumulation (LAMA).

References for Supplementary Information:

1. Q. Du, A. Kotlyar and A. Vologodskii, *Nucleic acids research*, 2008, **36**, 1120-1128.
2. J. P. Laine, P. L. Opresko, F. E. Indig, J. A. Harrigan, C. von Kobbe and V. A. Bohr, *Cancer Res*, 2003, **63**, 7136-7146.