

# SCIENTIFIC REPORTS



OPEN

## Fluorescently labeled circular DNA molecules for DNA topology and topoisomerases

Maxwell Gu<sup>1,2</sup>, Andrea Berrido<sup>1,2</sup>, Walter G. Gonzalez<sup>1,2</sup>, Jaroslava Miksovská<sup>1,2</sup>, Jeremy W. Chambers<sup>1,3</sup> & Fenfei Leng<sup>1,2</sup>

Received: 20 July 2016

Accepted: 10 October 2016

Published: 31 October 2016

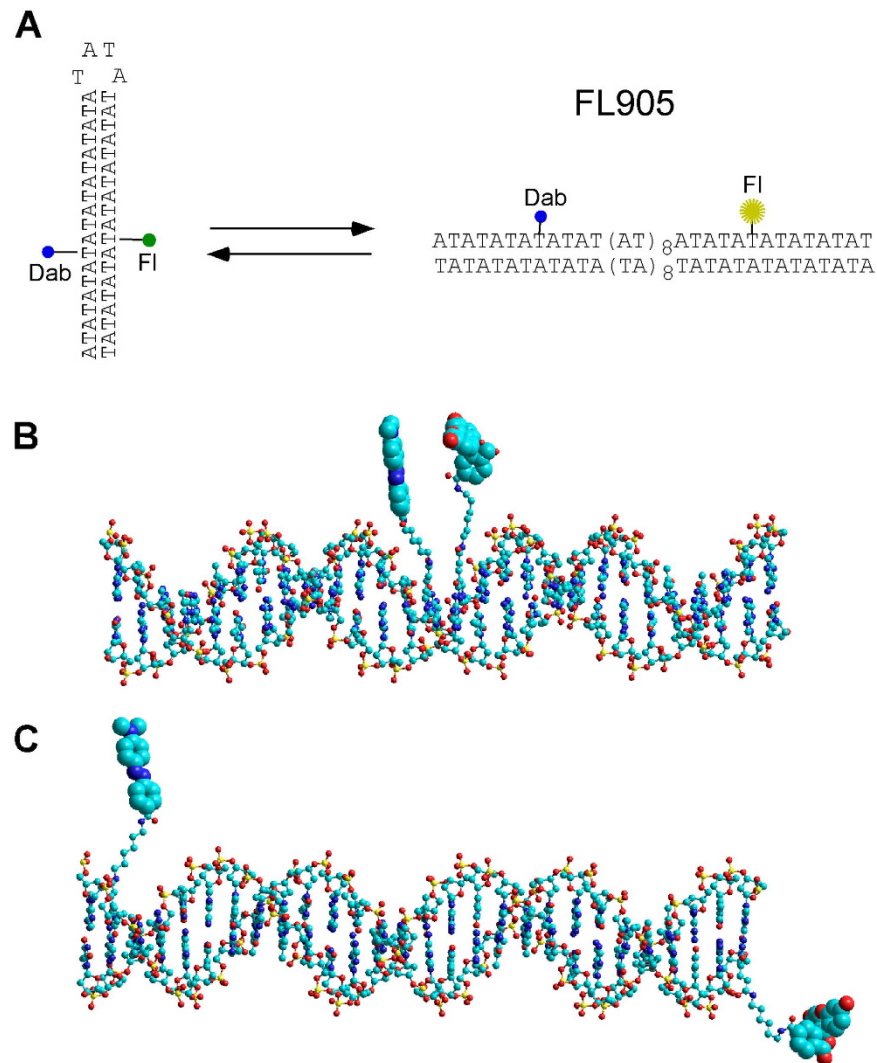
DNA topology plays essential roles in several fundamental biological processes, such as DNA replication, recombination, and transcription. Typically agarose gel electrophoresis is employed to study DNA topology. Since gel electrophoresis is time-consuming and labor intensive, it is desirable to develop other methods, such as fluorescence-based methods, for such studies. In this paper we report the synthesis of a type of unique fluorescence-labeled DNA molecules that can be used to study DNA topology and topoisomerases by fluorescence resonance energy transfer (FRET). Specifically, we inserted an 82 nt. synthetic DNA oligomer FL905 carrying a 42 nt. AT sequence with fluorescein and dabcyI labels into a gapped DNA molecule to generate relaxed and supercoiled pAB1\_FL905. Since the fluorescence intensity of pAB1\_FL905 is dependent on its supercoiling status, pAB1\_FL905 is a powerful tool to study DNA topology and topoisomerases by FRET. pAB1\_FL905 can also be developed into rapid and efficient high-throughput screening assays to identify inhibitors that target various DNA topoisomerases.

DNA supercoiling is a fundamental property of chromosomal DNA and plays critical roles in many essential DNA transactions, such as transcription, DNA replication, and recombination<sup>1,2</sup>. Usually agarose gel electrophoresis is used to study DNA supercoiling. Since gel electrophoresis is time-consuming and labor intensive, it is desirable to develop other assays, such as fluorescence-based assays, to study DNA topology and topoisomerases. For example, fluorescence dyes, such as PicoGreen<sup>3</sup>, have been shown to differentially bind to supercoiled (sc) and relaxed (rx) DNA molecules to yield different fluorescence properties. These fluorescence dyes were used to study DNA topoisomerases. However, the difference of the fluorescence intensity of the dyes binding to sc and rx DNA is too small to be widely used to study properties of DNA topoisomerases and to screen inhibitors against these topoisomerases<sup>3</sup>.

Another type of assays were developed from the utility of a unique property of sc DNA molecules that prefer binding to triplex-form oligomers if the sc plasmids contain one or multiple triplex-forming sequences<sup>4,5</sup>. Maxwell and coworkers invented a method in which an immobilized triplex-forming oligomer more efficiently captures sc plasmids than rx plasmids<sup>4</sup>. The captured plasmids can be subsequently quantified by a DNA-binding dye, such as SYBR Green. However, this method requires immobilization of oligomer to a solid surface, filtration, and multiple washing steps. Since streptavidin-coated 1536-well plates are not commercially available, this method is not compatible with ultra-high throughput screening to identify gyrase inhibitors from small compound libraries using 1536-well plates. Another method, also based on the triplex-forming oligomers, was developed by using fluorescence anisotropy for the readout<sup>5</sup>. Nevertheless, the signal to noise ratio is a concern and an expensive fluorimeter with the capacity to measure fluorescence anisotropy is required<sup>5</sup>.

More recently, Berger and coworkers made a circular plasmid DNA template that contains a fluorophore (fluorescein) and quencher (dabcyI), and developed a real-time assay to study DNA topological changes with this fluorescently labeled DNA<sup>6</sup>. However, the production yield of the fluorescently labeled DNA was too low to allow the assay to be widely used<sup>6</sup>. Additionally, because of the low yield of the DNA substrate, it makes the assay too costly. Here we describe a method to produce a type of fluorescently labeled circular DNA molecules with high yields to study DNA topology and topoisomerases by fluorescence resonance energy transfer (FRET). We also

<sup>1</sup>Biomolecular Science Institute, Florida International University, Miami, FL 33199. <sup>2</sup>Department of Chemistry & Biochemistry, Florida International University, Miami, FL 33199. <sup>3</sup>Department of Cellular Biology and Pharmacology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL 33199. Correspondence and requests for materials should be addressed to F.L. (email: lengf@fiu.edu)

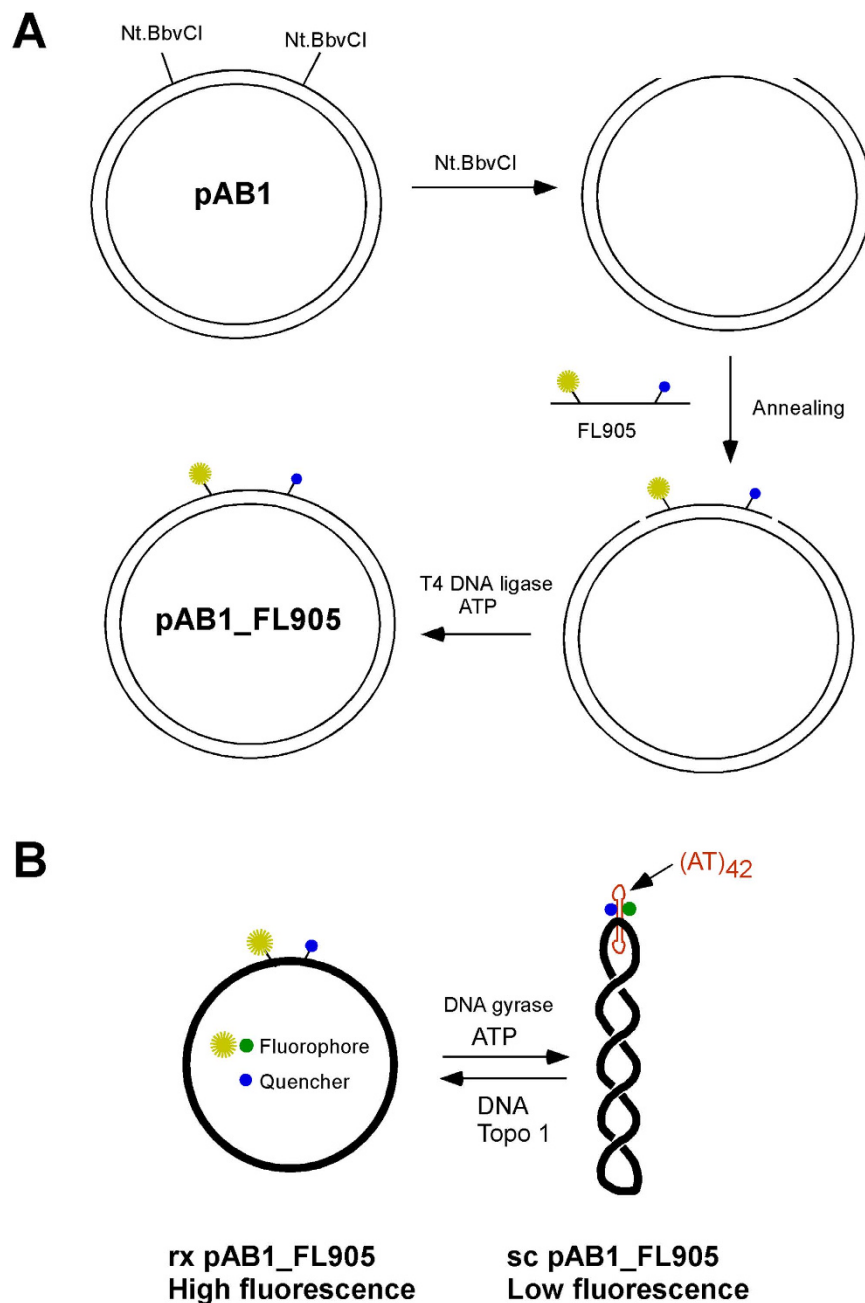


**Figure 1. Molecular models of  $(AT)_{42}$  DNA carrying fluorescein (Fl) and dabcy1 (Dab) labels. (A)** The 42 nt AT sequence of FL905 can convert from a hairpin structure to an open structure. The fluorescence of Fl is quenched by Dab in the hairpin structure. **(B)** The Fl and Dab labels are in proximity to each other when the  $(AT)_{42}$  of FL905 adopts the hairpin structure. **(C)** The positions of the Fl and Dab labels are far away when the  $(AT)_{42}$  sequence is in the double stranded state.

demonstrate that these unique DNA molecules can be used to screen anti-cancer drugs and antibiotics targeting DNA topoisomerases.

## Results and Discussion

**Experimental strategies to construct relaxed (rx) and supercoiled (sc) pAB1\_FL905.** As demonstrated previously<sup>7-9</sup>, (-) supercoiling induces localized DNA conformation transitions, such as cruciform formation of inverted repeat sequences. These topology-dependent, structural isomerizations could be used to gauge the superhelicity of the DNA molecules. As shown by Lilley *et al.*<sup>10</sup> and Mirkin *et al.*<sup>11-14</sup>, alternating adenine-thymine sequences  $[(AT)_n]$  undergo very rapid cruciform formation, as no detectable kinetic barrier prevents rapid interconversion between extruded and unextruded conformations in sc plasmid DNA templates<sup>10</sup>. We therefore decided to utilize this property of  $[(AT)_n]$  to monitor supercoiling change of plasmid DNA templates. We designed an 82 nt DNA oligomer FL905 that contains a dabcy1-labeled dT at 29th position from the 5'-end (the 8<sup>th</sup> position of the AT sequence from the 5' end; Fig. 1A) and a fluorescein-labeled dT at 55th position from the 5'-end (the 34<sup>th</sup> position of the AT sequence from the 5' end, Fig. 1A). Figure S1A shows that FL905 has intrinsic fluorescence before EB staining. We reasoned that if the 42 nt AT sequence adopts the hairpin structure, both the fluorescein and dabcy1 are in close proximity in the major groove ( $\sim 20$  Å; Fig. 1B). The fluorescence of fluorescein should be greatly quenched. In contrast, when the 42 nt AT sequence adopts the double-stranded DNA form, the distance between the fluorescein and dabcy1 should be more than 88.4 Å for B-form DNA ( $26 \text{ bp} \times 3.4 \text{ Å} = 88.4 \text{ Å}$ ). Indeed, our molecular model shows that the distance between the fluorescein and dabcy1 is  $\sim 100$  Å (Fig. 1C). The fluorescence of fluorescein should not be quenched. Figure S1B shows a fluorescence



**Figure 2.** An experimental strategy to construct relaxed (rx) or supercoiled (sc) pAB1\_FL905. (A) Oligomer FL905 that contains the 42 nt. AT sequence is ligated between the two Nt.BbvCI sites of plasmid pAB1 to yield rx pAB1\_FL905. (B) Sc pAB1\_FL905 can be generated through the treatment of rx pAB1\_FL905 by *E. coli* DNA gyrase. The fluorescence intensity of fluorescein is dependent on the supercoiling status of pAB1\_FL905.

melting experiment in which a four-fold fluorescence intensity change of fluorescein was observed upon the 42 nt AT hairpin structure was melted. This result demonstrates that fluorescence resonance energy transfer (FRET)<sup>15</sup> can be used to study the interconversion between extruded and unextruded conformations of FL905.

Next, we constructed pAB1 by inserting a synthetic double-stranded oligomer FL\_AT42, which carries the 42 bp AT sequence (Figure S1C), between the SphI and BamHI sites of pUC18 (Figure S1D). Plasmid pAB1 also contains two nicking endonuclease Nt.BbvCI recognition sites in the same orientation. In this way, DNA oligomer FL905 can be inserted between the two Nt.BbvCI sites according to a strategy described in Fig. 2A (a similar strategy was used to study DNA recombination by Levene and coworkers<sup>16</sup> and label large DNA fragments<sup>17,18</sup>). Because rx pAB1\_FL905 is the only theoretical ligation product, the production yield should be near 100%. Sc pAB1\_FL905 can be generated through the treatment of rx pAB1\_FL905 by bacterial DNA gyrase in the presence of ATP (Fig. 2B). Rx and sc pAB1\_FL905 should be powerful tools to study DNA topology and topoisomerases by FRET.

**Fluorescence properties of relaxed, nicked, supercoiled pAB1\_FL905.** To date, we have produced ~0.5 mg of rx pAB1\_FL905 and ~0.6 mg of sc pAB1\_FL905 using the strategy described in Fig. 2 and purified by CsCl-EB equilibrium gradient banding with approximately 60% yield. A detailed procedure was provided in “Materials and Methods” and also in Figure S2. Figure S3 shows gel images at various stages of the procedure. As expected, rx and sc pAB1\_FL905 have intrinsic fluorescence before EB staining (Figure S3D).

After we obtained rx and sc pAB1\_FL905, we compared fluorescence properties of sc, rx, and nicked (nk) pAB1\_FL905. Figure 3A shows our results. As expected, the fluorescence intensity of rx or nk pAB1\_FL905 is significantly higher than that of the sc pAB1\_FL905. Figure 3B–D show kinetic results of pAB1\_FL905 reacting with three different enzymes: Nt.BbvCI, *E. coli* topoisomerase I, and *E. coli* DNA gyrase. As anticipated, Nt.BbvCI was able to quickly nick sc pAB1\_FL905 with a half-life of ~15 seconds (Fig. 3B). This result supports previous conclusions regarding (AT)<sub>n</sub> that undergoes very rapid cruciform formation, as no detectable kinetic barrier prevents rapid interconversion between extruded and unextruded conformations in sc plasmid DNA templates<sup>10</sup>. It also suggests that pAB1\_FL905 is a good DNA substrate to study DNA topology and topoisomerases by FRET. Similarly, large amounts of *E. coli* DNA topoisomerase I was able to rapidly relax sc pAB1\_FL905 (Fig. 3C). The kinetics of *E. coli* DNA gyrase was relatively slow (Fig. 3D). Nevertheless, further studies are required to determine kinetic parameters of these enzymes.

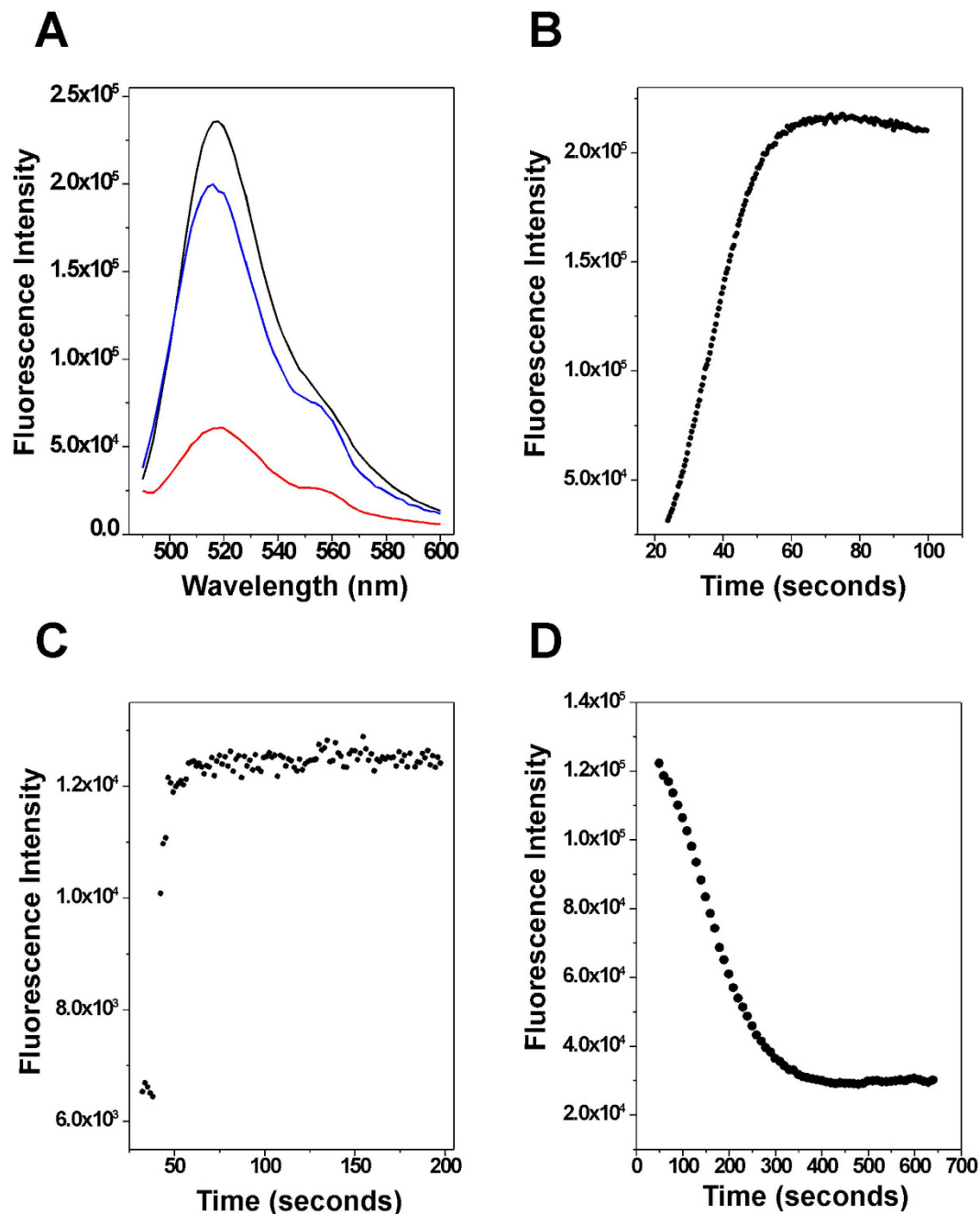
We also designed three similar fluorescence-labeled oligomers. FL919 and FL920 contain a dabcy1-labeled dT at 27th and 31<sup>st</sup> position, and a fluorescein-labeled dT at 57th and 53th position from the 5'-end, respectively. In other words, the distance between the dabcy1 and fluorescein is different for FL905, FL919, and FL920. FL924 carries a BHQ2-labeled dT at 29th position from the 5'-end (the 8<sup>th</sup> position of the AT sequence from the 5'-end; Figure S5) and a TAMRA-labeled dT at 55th position from the 5'-end (the 34<sup>th</sup> position of the AT sequence from the 5'-end, Figure S5). These three oligomers were inserted between the two Nt.BbvCI sites of pAB1 to yield rx and sc pAB1\_FL919, pAB1\_FL920, and pAB1\_FL924. Figures S4 and S5 show fluorescence properties of these DNA molecules. Similar to pAB1\_FL905, the fluorescence intensity of rx pAB1\_FL919, pAB1\_FL920, and pAB1\_FL924 is significantly higher than that of the sc DNA molecules (Figures S4 and S5). However, the FRET efficiency of pAB1\_FL919 and pAB1\_FL920 is lower than that of pAB1\_FL905. This result demonstrated that FL905 has the optimal distance between dabcy1 and fluorescein for studying supercoiling-dependent transitions of pAB1 by FRET. As expected, the fluorescence intensity of pAB1\_FL924 is lower than that of pAB1\_FL905 although the FRET efficiency is similar for both DNA molecules.

**Potential applications.** Rx or sc fluorescently labeled pAB1\_FL905 or similar DNA molecules should have many potential applications. As shown above, they can be used to study supercoiling-dependent DNA topological changes or determine biochemical properties and kinetics of various DNA topoisomerases. Nevertheless, in our opinion, the most important application of these DNA molecules is to screen inhibitors or compounds targeting different DNA topoisomerases since many of these compounds are either anticancer drugs, such as doxorubicin<sup>19</sup>, or antibiotics, such as ciprofloxacin<sup>20</sup>. Figure S6A shows a strategy for identifying bacterial DNA gyrase inhibitors. In the absence of gyrase inhibitors, bacterial DNA gyrase is capable of converting rx DNA templates into sc DNA molecules. As demonstrated above, this conversion results in quenching of fluorescence of pAB1\_FL905. However, DNA gyrase inhibitors should inhibit this conversion. In this way, the fluorescence intensity of rx pAB1\_FL905 should not be changed. A titration experiment should also yield an inhibition IC<sub>50</sub> for the gyrase inhibitor. According to this strategy, we performed titration experiments in which different concentrations of novobiocin and ciprofloxacin were added into DNA supercoiling assays. Figure 4 shows our results. Novobiocin and ciprofloxacin potently inhibited the activities of DNA gyrase with an estimated IC<sub>50</sub> of 0.48 ± 0.14 and 2.57 ± 1.62 μM, respectively. Agarose gel electrophoresis confirmed that these antibiotics indeed potently inhibited DNA gyrases activities (Figure S6B). Due to simplicity, this FRET assay can be easily adapted into a high throughput format to identify gyrase inhibitors from millions of compounds found in small molecule libraries. Similar assays may be used to identify inhibitors targeting other DNA topoisomerase, such human DNA topoisomerase I and II. Furthermore, fluorescently labeled sc or rx plasmid DNA molecules may be used to study DNA recombination<sup>16</sup> or other supercoiling-dependent structural transitions, such as supercoiling-induced G-quadruplex<sup>21</sup>.

In summary, we have invented a novel method to produce a type of fluorescence-labeled rx or sc DNA molecule, such as pAB1\_FL905 in the milligram range. This type of DNA molecules could be developed into powerful tools to study DNA topology and topoisomerases. Since only a few nano grams of pAB1\_FL905 are needed for 384-well or 1536-well plates for detection, these DNA molecules could be used to develop rapid and efficient high-throughput screening assays to identify inhibitors from the millions of compounds found in small molecule libraries that may target DNA topoisomerases, such as bacterial DNA gyrase and human DNA topoisomerase I & II.

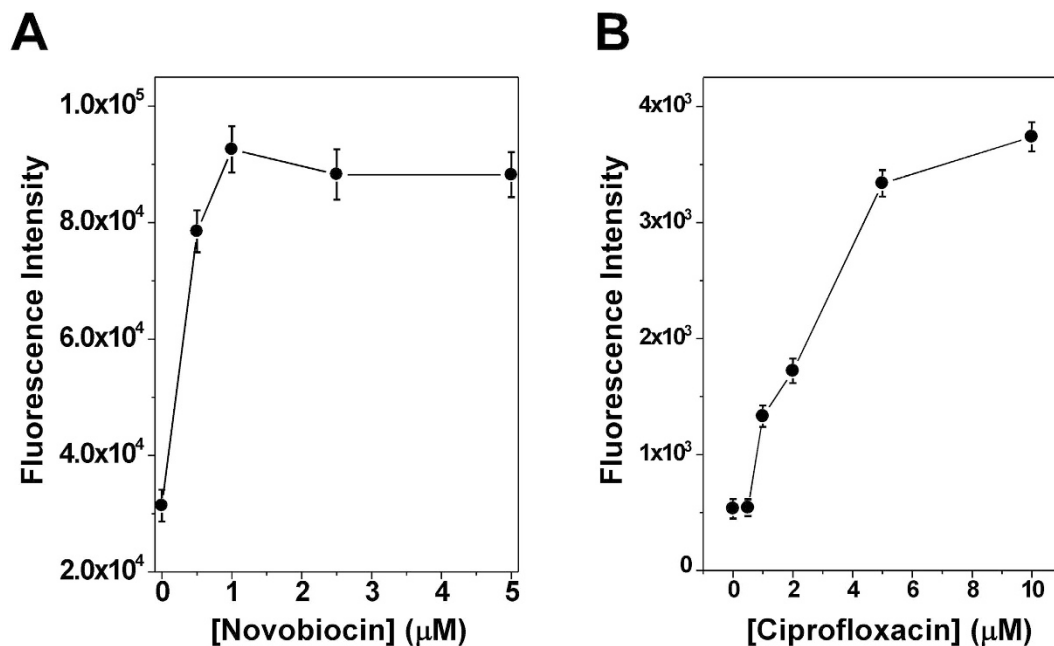
## Methods

**Materials.** Restriction enzymes Nt.BbvCI, SphI, BamHI, *E. coli* DNA gyrase, and T4 DNA ligase were purchased from New England Biolabs (Beverly, MA, USA). *E. coli* DNA topoisomerase I was purified as described previously<sup>22</sup>. The following synthetic oligonucleotides were purchased from MWG-Biotech, Inc. (Huntsville, AL): FL882 (5'-CCCTCAGCCCACAGCAGACGATGGGCCAACCAACCAGCCCC-3'), FL883 (5'-GATCCCGTGAGGGCTGGTTGGTTGGCCCATCGTCTCGTGCTGTCGGGCTGAGGGCATG-3'), FL905 (5'-TCAGCCCGACAGCACGAGACGATATATA[Dab-dT]ATATATATATATATATATATATATA[Fl-dT]ATATATATGCGGCAACCAACCAGCCCC-3'), FL919 (5'-TCAGCCCGACAGCACGAGACGATATA[Dab-dT]ATATATATATATATATATATATATATATATATA[Fl-dT]ATATATGCGGCAACCAACCAGCCCC-3'), FL920 (5'-TCAGCCCGACAGCACGAGACGATATATA[Dab-dT]



**Figure 3.** (A) Fluorescence spectra of sc (red line), rx (black line), and nk (blue line) pAB1\_FL909.  $\lambda_{ex} = 470$  nm. (B) Kinetics of the nicking reaction by Nt.BbvCI. Briefly,  $60 \mu\text{L}$  of  $1 \times$  CutSmart buffer containing  $500 \text{ ng}$  of sc pAB1\_FL905 was prepared and equilibrated to  $37^\circ\text{C}$ .  $20$  units of Nt.BbvCI were added to initiate the nicking reaction. The fluorescence intensity at  $\lambda_{em} = 521 \text{ nm}$  was monitored with  $\lambda_{ex} = 470 \text{ nm}$ . (C) Kinetics of the relaxation reaction by *E. coli* DNA topoisomerase I. For the relaxation reaction,  $90 \mu\text{L}$  of  $1 \times$  NEBuffer 4 ( $50 \text{ mM}$  KAc,  $20 \text{ mM}$  Tris-Ac,  $10 \text{ mM}$   $\text{Mg}(\text{AC})_2$ ,  $1 \text{ mM}$  DTT, pH 7.9) containing  $270 \text{ ng}$  of sc pAB1\_FL905 was prepared and equilibrated to  $37^\circ\text{C}$ .  $0.67 \mu\text{M}$  of *E. coli* DNA topoisomerase I was used to relax the sc pAB1\_FL905. The fluorescence intensity at  $\lambda_{em} = 521 \text{ nm}$  was monitored with  $\lambda_{ex} = 470 \text{ nm}$ . (D) Kinetics of the supercoiling reaction by *E. coli* DNA gyrase. For the supercoiling reaction,  $90 \mu\text{L}$  of  $1 \times$  gyrase buffer containing  $1 \mu\text{g}$  of rx pAB1\_FL905 was prepared and equilibrated to  $37^\circ\text{C}$ .  $30$  units of *E. coli* DNA gyrase was used to supercoil the rx pAB1\_FL905. The fluorescence intensity at  $\lambda_{em} = 521 \text{ nm}$  was monitored with  $\lambda_{ex} = 470 \text{ nm}$ .

ATATATATATATATATATATA[Fl-dT]ATATATATATGGGCCCAACCAACCAGCCCC-3'), and FL924 (5'-TCAGCCCGACAGCAGGACGATATATA[BHQ2-dT]ATATATATATATATATATATATATA[TAM-dT]ATATATATGGGCCCAACCAACCAGCCCC-3') where Dab-dT, Fl-dT, BHQ2-dT, and TAM-dT represent dabcy1-dT,



**Figure 4.** DNA gyrase was potently inhibited by novobiocin (A) and ciprofloxacin (B). For DNA supercoiling reactions, 60 μL of 1 × gyrase buffer containing 670 ng of rx pAB1\_FL905 was prepared and equilibrated to 37 °C. 20 units of DNA gyrase was used to supercoil the rx pAB1\_FL905 in the presence of different concentrations of novobiocin and ciprofloxacin. The fluorescence intensity at  $\lambda_{em} = 521$  nm was monitored with  $\lambda_{ex} = 494$  nm. The inhibition IC<sub>50</sub> was estimated to be  $0.48 \pm 0.14$  and  $2.57 \pm 1.1$  μM for novobiocin and ciprofloxacin, respectively.

fluorescein-dT, BHQ2-dT, and TAMRA-dT, respectively. QIAquick Nucleotide Removal Kit and QIAquick Gel Extraction Kit were obtained from Qiagen, Inc (Valencia, CA).

**Plasmids.** Plasmid pAB1 (2,757 bp) was constructed by inserting a 95 bp synthetic DNA fragment FL\_AT42 (the annealing product of FL882 and FL883) between the SphI and BamHI sites of pUC18. DNA sequencing was used to verify the inserted DNA sequence.

**Synthesis of relaxed (rx) and supercoiled (sc) pAB1\_FL905, pAB1\_FL919, pAB1\_FL920, and pAB1\_FL924.** For a typical small scale of reaction, 10 μg of pAB1 (~5.7 pmol) was digested by 25 units of Nt.BbvCI in 200 μL of 1 × CutSmart Buffer (50 mM KAc, 20 mM Tris-Ac, 10 mM Mg(AC)<sub>2</sub>, 100 μg/mL BSA, pH 7.9). After the digestion, 80 pmol of phosphorylated FL905 was added into the reaction mixture. The reaction mixture was incubated at 90 °C in a 4-liter water bath for one minute and then cooled down to room temperature in the water bath (~4 to 5 hours; usually this step was carried out overnight). To generate rx pAB1\_FL905, 300 units of T4 DNA ligase were added into the reaction mixtures in the presence of 10 mM of DTT and 2 mM of ATP (final concentrations). The reaction mixtures were incubated at 37 °C to seal the nicks and yield rx pAB1\_FL905. The rx pAB1\_FL905 was separated by 1% agarose gel electrophoresis and purified by QIAquick Gel Extraction Kit. Typically, we were able to obtain ~6 μg of rx pAB1\_FL905 (~60% yield). To produce sc pAB1\_FL905, 1 μg of rx pAB1\_FL905 was treated with 5 units of *E. coli* DNA gyrase for 1 hour at 37 °C. The sc pAB1\_FL905 can be purified by QIAquick Nucleotide Removal Kit or separated by 1% agarose gel and purified by QIAquick Gel Extraction Kit. An alternative procedure was also used to produce sc pAB1\_FL905. First, the annealed product of the Nt.BbvCI digested pAB1 and FL905 was purified by QIAquick Nucleotide Removal Kit. The purified DNA sample (~1 μg) was ligated with 300 units of T4 DNA ligase in the presence of 5 units of DNA gyrase. The sc and rx pAB1\_FL905 were separated by using a 1% agarose gel and purified by using QIAquick Gel Extraction Kit. Rx and sc pAB1\_FL919, pAB1\_FL920, and pAB1\_FL924 were also generated similarly.

For a typical large scale of reaction, 1 mg of pAB1 (~570 pmol) was digested by 2,500 units of Nt.BbvCI in 20 mL of 1 × CutSmart Buffer for one hour at 37 °C. After the digestion, 8,000 pmol of phosphorylated FL905 was added into the reaction mixture. The reaction mixture was incubated at 90 °C in a 4-liter water bath for two minutes and then cooled down to room temperature in the water bath (~4 to 5 hours; usually this step was carried out overnight). To generate rx pAB1\_FL905, 25,000 units of T4 DNA ligase were added into the reaction mixtures in the presence of 10 mM of DTT and 2 mM of ATP (final concentrations). The reaction mixtures were incubated at 37 °C to seal the nicks and yield the relaxed pAB1\_FL905. The unpurified rx pAB1\_FL905 sample was extracted with 20 mL of phenol, precipitated with ethanol, and washed once with 70% ethanol. Rx pAB1\_FL905 was purified by CsCl-EB equilibrium gradient banding. A total of 486 μg of rx pAB1\_FL905 was produced by this procedure. To generate sc pAB1\_FL905, the ligation reaction was carried out in the presence of 25 μM of ethidium bromide. The unpurified sc pAB1\_FL905 sample was extracted twice with 20 mL of phenol, precipitated

with ethanol, and washed once with 70% ethanol. Sc pAB1\_FL905 was purified by CsCl-EB equilibrium gradient banding. Alternatively, after phenol extraction and ethanol precipitation, the unpurified rx pAB1\_FL905 sample was treated with *E. coli* DNA gyrase in the presence of 2 mM of ATP at 37 °C for one hour to yield sc pAB1\_FL905. Sc pAB1\_FL905 was purified by CsCl-EB equilibrium gradient banding. A total of 571 µg of sc pAB1\_FL905 was obtained.

**Fluorescence spectroscopy.** Fluorescence measurements were performed using an ISS, Inc., PC1 photo counting spectrofluorimeter with an excitation wavelength of 470 nm and bandwidth resolution of ±4 nm or a Biotek Synergy H1 Hybrid Plate Reader with an excitation wavelength of 482 nm.

**Gyrase inhibition assay.** DNA gyrase inhibition assays were performed in 50 µL of 1× gyrase buffer (35 mM Tris-HCl, 24 mM KCl, 4 mM MgCl<sub>2</sub>, 2 mM DTT, 1.75 mM ATP, 5 mM spermidine, 0.1 mg/mL BSA, 6.5% glycerol, pH7.5) containing 560 ng of rx pAB1\_FL905 and equilibrated to 37 °C. 20 units of DNA gyrase was used to supercoil the rx pAB1\_FL905 in the presence of different concentrations of novobiocin and ciprofloxacin. The fluorescence intensity at λ<sub>em</sub> = 521 nm was monitored with λ<sub>ex</sub> = 494 nm in a microplate reader. The IC<sub>50</sub> values were estimated by nonlinear fitting of the following equation:  $F = F_{min} + \frac{F_{max} - F_{min}}{1 + 10^{(\log(IC_{50}) - x)P}}$  where F is the fluorescence intensity at the x concentration of an inhibitor. F<sub>max</sub> and F<sub>min</sub> are the maximum and minimum fluorescence of the DNA sample, respectively. P is a slope parameter.

**Molecular Modeling.** DNA molecular models are generated using HyperChem 8.0.

## References

- Bates, A. D. & Maxwell, A. *DNA Topology* (Oxford University Press, Oxford, UK, 2005).
- James, C. *Wang Untangling the Double Helix: DNA Entanglement and the Action of the DNA Topoisomerases* (Cold Spring Harbor Laboratory Press, 2008).
- Roychoudhury, S. *et al.* Development and use of a high-throughput bacterial DNA gyrase assay to identify mammalian topoisomerase II inhibitors with whole-cell anticancer activity. *J. Biomol. Screen.* **8**, 157–163 (2003).
- Maxwell, A., Burton, N. P. & O'Hagan, N. High-throughput assays for DNA gyrase and other topoisomerases. *Nucleic Acids Res.* **34**, e104 (2006).
- Shapiro, A. *et al.* A homogeneous, high-throughput fluorescence anisotropy-based DNA supercoiling assay. *J. Biomol. Screen.* **15**, 1088–1098 (2010).
- Jude, K. M., Hartland, A. & Berger, J. M. Real-time detection of DNA topological changes with a fluorescently labeled cruciform. *Nucleic Acids Res.* **41**, e133 (2013).
- Rahmouni, A. R. & Wells, R. D. Direct evidence for the effect of transcription on local DNA supercoiling *in vivo*. *J. Mol. Biol.* **223**, 131–144 (1992).
- Lilley, D. M. The inverted repeat as a recognizable structural feature in supercoiled DNA molecules. *Proc. Natl. Acad. Sci. USA* **77**, 6468–6472 (1980).
- Panayotatos, N. & Wells, R. D. Cruciform structures in supercoiled DNA. *Nature* **289**, 466–470 (1981).
- Greaves, D. R., Patient, R. K. & Lilley, D. M. Facile cruciform formation by an (A-T)<sub>34</sub> sequence from a *Xenopus* globin gene. *J. Mol. Biol.* **185**, 461–478 (1985).
- Panyutin, I., Lyamichev, V. & Mirkin, S. A structural transition in d(AT)n.d(AT)n inserts within superhelical DNA. *J. Biomol. Struct. Dyn.* **2**, 1221–1234 (1985).
- Dayn, A. *et al.* Formation of (dA-dT)n cruciforms in *Escherichia coli* cells under different environmental conditions. *J. Bacteriol.* **173**, 2658–2664 (1991).
- Dayn, A., Malkhosyan, S. & Mirkin, S. M. Transcriptionally driven cruciform formation *in vivo*. *Nucleic Acids Res.* **20**, 5991–5997 (1992).
- Krasilnikov, A. S., Podtelezchnikov, A., Vologodskii, A. & Mirkin, S. M. Large-scale effects of transcriptional DNA supercoiling *in vivo*. *J. Mol. Biol.* **292**, 1149–1160 (1999).
- Vitoc, C. I. & Mukerji, I. HU binding to a DNA four-way junction probed by Forster resonance energy transfer. *Biochemistry* **50**, 1432–1441 (2011).
- Shoura, M. J. *et al.* Measurements of DNA-loop formation via Cre-mediated recombination. *Nucleic Acids Res.* **40**, 7452–7464 (2012).
- Luzzietti, N., Knappe, S., Richter, I. & Seidel, R. Nicking enzyme-based internal labeling of DNA at multiple loci. *Nat. Protoc.* **7**, 643–653 (2012).
- Luzzietti, N. *et al.* Efficient preparation of internally modified single-molecule constructs using nicking enzymes. *Nucleic Acids Res.* **39**, e15 (2011).
- Tewey, K. M., Rowe, T. C., Yang, L., Halligan, B. D. & Liu, L. F. Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. *Science* **226**, 466–468 (1984).
- Collin, F., Karkare, S. & Maxwell, A. Exploiting bacterial DNA gyrase as a drug target: current state and perspectives. *Appl. Microbiol. Biotechnol.* **92**, 479–497 (2011).
- Sun, D. & Hurlley, L. H. The importance of negative superhelicity in inducing the formation of G-quadruplex and i-motif structures in the c-Myc promoter: implications for drug targeting and control of gene expression. *J. Med. Chem.* **52**, 2863–2874 (2009).
- Xu, X. & Leng, F. A rapid procedure to purify *Escherichia coli* DNA topoisomerase I. *Protein Expr. Purif.* **77**, 214–219 (2011).

## Acknowledgements

This work was supported by grant 1R15GM109254-01A1 from the National Institutes of Health (to F.L.).

## Author Contributions

F.L. designed research; M.G., A.B., W.G.G. and F.L. performed research; F.L. constructed the molecular models; F.L., J.M. and J.W.C. analyzed data; F.L. wrote the paper.

## Additional Information

**Supplementary information** accompanies this paper at <http://www.nature.com/srep>

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Gu, M. *et al.* Fluorescently labeled circular DNA molecules for DNA topology and topoisomerases. *Sci. Rep.* **6**, 36006; doi: 10.1038/srep36006 (2016).

**Publisher's note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>

© The Author(s) 2016