Tricking Enzymes In Living Cells: A Mechanism-Based Strategy For Design of DNA Topoisomerase Biosensors

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Abstract

Most activity-based molecular probes are designed to target enzymes that catalyze the breaking of chemical bonds and the conversion of a unimolecular substrate into bimolecular products. However, DNA topoisomerases are a class of enzymes that alter DNA topology without producing any molecular segments during catalysis, which hinders the development of practical methods for diagnosing these key biomarkers in living cells. Here, we established a new strategy for the effective sensing of the expression levels and catalytic activities of topoisomerases in cell-free systems and human cells. Using our newly designed biosensors, we tricked DNA topoisomerases within their catalytic cycles to switch on fluorescence and resume new rounds of catalysis. Considering that human topoisomerases have been widely recognized as biomarkers for multiple cancers and identified as promising targets for several anticancer drugs, we believe that these DNA-based biosensors and our design strategy would greatly benefit the future development of clinical tools for cancer diagnosis and treatment.

Introduction

The catalytic action of most cellular enzymes involves the breaking of chemical bonds and the conversion of a unimolecular substrate into bimolecular products (Figure S1a).\(^1\) These intrinsic properties of enzymes have been extensively utilized in the past to design fluorescent probes for sensing enzymatic behaviors in cells (Figure S1b).\(^2\)–\(^5\) For example, a peptide derivative with a caspase cleavage site and a fluorophore-quencher pair was recently synthesized.\(^4\) As a result of its exclusive response to the catalytic action of caspase (Figure S2), this peptide derivative was reported as a biosensor to quantify caspases in biological samples.\(^4\) Another example is an iminocoumarin-benzothiazole-based structure with a covalently linked phosphate group.\(^5\) Once the cellular alkaline phosphatase breaks the phosphate ester bond within the molecule (Figure S3), quenched fluorescence is restored in living cells.\(^5\) In addition to the aforementioned examples, a wide variety of enzymes have been detected using molecular probes designed on the basis of unimolecular to bimolecular conversion processes.\(^6\)–\(^9\)

In contrast to enzymes that catalyze the breaking of chemical bonds,\(^2\)–\(^9\) DNA topoisomerases change the topological state of DNA molecules without releasing any molecular segments.\(^10,\,11\) More specifically, the connectivity among the atoms in the enzyme substrate is identical to that in its products (Figure S1c), which hinders the design of fluorescent probes using conventional bimolecular formation approaches. Even though the detection of some of the enzymes in cell-free systems has been attempted recently,\(^12\)–\(^15\) the specific enzymatic action of DNA topoisomerases explains the lack of cellular biosensors targeting these key enzymes in living cells nowadays.

Here, we report a novel strategy for diagnosing cellular DNA topoisomerases, enzymes whose natural substrates and products have identical molecular configurations. To demonstrate the viability of this strategy, proof-of-concept studies were performed using human topoisomerase I (topo I) as the target. Using our newly designed mechanism-based biosensors, we tricked DNA topoisomerases twice during
catalysis (Fig. 1). First, the enzyme is misled to act on a specifically designed pseudo-substrate oligonucleotide and consequently switch on the quenched fluorescence from a DNA-based probe. Then, topo I is tricked into accepting an oligonucleotide moiety disguised as part of its original substrate to proceed with the subsequent elimination process. This action allows topo I to effectively detach from the covalent conjugate and resume its original structure for new rounds of catalysis. In principle, this mechanism-based strategy, with certain adaptations, should be suitable for designing molecular probes for DNA topoisomerases and other cellular enzymes that cause no changes in atomic connectivity during catalysis.

From a clinical point of view, human topoisomerase I is overexpressed in a variety of human cancer cells and has been identified as a key biomarker for cancer diagnosis, prognosis, and monitoring therapeutic responses.\[^{16-22}\] Moreover, it serves as a cellular target for several FDA-approved anticancer drugs.\[^{23, 24}\] In this study, we demonstrate that our DNA-based biosensors can effectively sense differences in topo I catalytic activity among human cancer cells, normal cells, topo I gene-knockdown cells, and chemotherapy agent-treated cells. Considering that topo I is a validated biomarker for a wide range of cancers and there are no molecular tools available thus far for diagnosing topo I expression levels in living cells, we believe that our newly designed probes could have great potential for developing clinical tools for determining cancer aggressiveness,\[^{20}\] monitoring cancer treatment,\[^{21, 22}\] and evaluating an individual’s predisposition for cancer.\[^{20}\]

**Results And Discussion**

2.1. Strategy for Designing Mechanism-Based Biosensors for DNA Topoisomerases

Topoisomerases change DNA supercoiling by initially binding to any sequence of double-stranded DNA.\[^{25-27}\] Westergaard group reported in 1985, however, human topoisomerase I does not act on all DNA sequences indiscriminately but preferentially binds to particular tracts (e.g., Duplex 1 shown in Figure 1a and 1b) in the macronuclear DNA of the eukaryote, Tetrahymena thermophila.\[^{28}\] Since then, these particular DNA sequences have been extensively studied,\[^{29-32}\] including the incorporation of such sequences into circular plasmids to facilitate DNA relaxation (Figure S4).\[^{33, 34}\] Taking advantage of the aforementioned discoveries,\[^{28-32}\] we designed a DNA-based biosensor in our study (Probe 1 in Figure 1c), which possesses the following characteristics:

(a) **Functional components incorporated in a unimolecular substrate-based oligonucleotide.** In comparison with Duplex 1, Probe 1 is a derived version of the topo I substrate that possesses five additional components in its structure (Fig. 1c): (i) a fluorophore (Cy3) covalently modified near the topo I binding site, (ii) a fluorescence quencher (BHQ-2) covalently modified at the 3’ terminus, (iii) a broken end with a 5’-flapped trinucleotide near the topo I cutting site, (iv) two stable hairpin loops, and (v) phosphorothioate modifications in phosphate backbones. To ensure that the modified oligonucleotide is still accessible to topo I, all modifications are incorporated at the sites where topo I is not in physical
contact. Most importantly, our DNA-based biosensor is a self-assembled unimolecular structure that can, in principle, enhance its stability in living cells.

**(b) Fluorescence quenching prior to the action of topo I.** Within the self-folding structure of Probe 1, a pair of fluorophore and fluorescence quencher merge in close proximity, which ensures that the adjacent BHQ-2 effectively quenches the fluorescence signals of Cy3 on the opposite strand.

**(c) Restoration of fluorescence induced by the catalytic action of topo I.** Once topo I cleaves the phosphodiester bond at its specific site, this enzyme is expected to covalently link to the 3' end of DNA and hold the 5' end through physical interactions, as in its natural catalytic cycle. As a result, a short BHQ-2-linked trinucleotide (Fragment 1 in Fig. 1e) is anticipated to depart from the main body of the probe, which will lead to the restoration of the Cy3 fluorescence signal. Thus, topo I is tricked into accepting a specially designed oligonucleotide as its substrate and initializing the dissociation of fluorophore-quencher pairs.

**(d) Recovery of topo I from the enzyme-substrate complex for new rounds of catalysis.** A flapped trinucleotide was designed at the 5' end of Probe 1 to fill the gap generated by the action of topo I. Once the flap structure fills the gap through Watson-Crick base pairing, topo I is predicted to ligate the free 5' end with the covalently linked 3' end of DNA. In this process, topo I is tricked for the second time to take the 5'-flapped trinucleotide as the departed fragment and to liberate itself from the enzyme-substrate complex for new rounds of catalysis.

**(e) Enhancement of intracellular stability with phosphorothioate linkages.** Phosphorothioate modifications were introduced at certain oligonucleotide positions (denoted with stars in Fig. 1c) to enhance its nuclease hydrolysis resistance. Furthermore, it should be noted that Probe 1 is always in equilibrium with its isoform (Fig. 1d). This equilibrium shifts to favor the formation of Probe 1 when it is consumed by the action of topo I in living cells. In addition to Probe 1, we designed other probes (Probe 2–7) for comparison purposes. Detailed nucleotide sequences and synthetic procedures for all probes are provided in the Supporting Information (Table S1, Figure S5 and S6). It has been demonstrated that Probe 1 exhibited the highest reactivity toward human topo I among all the probes (Figure S7) and was employed in our subsequent studies.

### 2.2. Validation of Our Newly Designed Biosensors in Cell-Free Systems

To determine whether the DNA-based biosensors are accessible and photoswitchable by topo I's catalytic action as originally designed, fluorescence spectroscopic examinations were conducted (Fig. 2). As seen in Fig. 2b and 2d, the fluorescence intensity was greatly induced by topo I treatment. In addition to these spectroscopic examinations, colorimetric studies also revealed that Probe 1, which was treated with topo I, yielded an orange color under UV irradiation (Fig. 2c and 2e). In separate studies, enhancement of the fluorescence intensity of Probe 1 was observed with increasing molar concentrations of topo I (Fig. 2f) and reaction time (Fig. 2g). In principle, topo I catalysis of Probe 1, low-molecular-weight DNA segments should be produced. To verify that the molecular structure of Probe 1 was indeed fragmented, a gel mobility shift assay was performed. As shown in Figure S8, with an increasing concentration of human
Most of the oligonucleotides were cleaved while lower molecular weight fragments were generated.

Even though Probes 2 and 3 closely resemble Probe 1 in their structures, none of these probes are designed to be the substrate of human topo I (Figure S9). Probe 2 is different from Probe 1 because it lacks a flap structure at its 5' end. As shown in the fluorescence spectra shown in Figure S9d, this probe could not generate fluorescence signals in the presence of topo I. This observation corroborates that the presence of 5' flap structures in Probe 1 is indispensable for recovering trapped topo I in its catalysis (Steps 4–6 in Fig. 1e). Additionally, Probe 3 was examined in our studies, whose structure was the same as Probe 1 except that a phosphate group was modified at its 5' end. As seen in Figure S9f, a negligible amount of fluorescence signal emerged upon incubation of Probe 3 with topo I, which is consistent with the suggestion that the phosphate at the 5' end of Probe 3 prevents the re-ligation reaction (Step 5 in Fig. 1e) of topo I from occurring. To investigate whether our newly designed probes are human topo I-specific, we examined the reactions of Probe 1 with E. coli topo I, human topo IIα, topo IV, DNA gyrase, and human topo I in parallel, which are all DNA supercoil-altering enzymes. As shown in Fig. 2h, all of these enzymes caused trivial enhancement of fluorescence signals except for human topo I, which is an indication that Probe 1 is highly specific to human topo I. All of the observations mentioned above are consistent with the notion that Probe 1 effectively induced human topo I in buffer solutions to switch on the quenched fluorescence.

2.3. Evaluation of the Performance of DNA-Based Biosensors in Cell Lysates

To determine how diverse types of intracellular proteins derived from human cells affect the DNA-based probes, protein lysates of ordinary HEK293T cells and topo I-upregulated HEK293T cells were treated separately with Probe 1. As shown in Fig. 2i, the protein lysates from ordinary cells led to the generation of low fluorescence signals, which corroborates that the proteins derived from these cells are incapable of causing effective chemical changes in Probe 1. In contrast to ordinary HEK293T cells, the protein lysates from topo I-upregulated HEK293T cells drove Probe 1 to produce significantly high fluorescence signals. These results suggest that our newly designed mechanism-based biosensors can be applied to purified analytes and human cell lysates containing overexpressed proteins.

2.4. Examination of the Responsiveness and Specificity of DNA-Based Biosensors in Living Cells

Upon validating their designed roles in buffer solutions and cell lysates, we subsequently examined whether these DNA-based biosensors could trick intracellular topoisomerases inside human living cells (Fig. 3). The human colon cancer cell line HT-29 was chosen for our subsequent studies, which is known to overexpress topo I in cellular environments. Probe 1 was introduced into living cells using liposomes as carriers (Fig. 3a) followed by confocal laser scanning microscopy. The results shown in Fig. 3b verified that intracellular topo I could effectively switch on the quenched fluorescence from Probe 1. These observations demonstrate that our newly designed DNA-based probes are compatible with cytoplasmic environments, especially in the presence of different types of metal ions, high concentrations...
of glutathione, and varied pH values. In other words, the cytoplasmic compatibility of these probes allows them to function as efficient substrates for intracellular topo I inside human cells.

In addition to Probe 1, HT-29 cells were incubated with Probe 2 during our investigations for comparison purposes (Fig. 3c). Due to the lack of a critical 5' flap, Probe 2 could not restore the fluorescence in living cells (Fig. 3d), which indicates that neither topo I nor other cellular enzymes could chemically dissociate the fluorophore-quencher pair in this probe. Since these two probes share similar structures except for a 5' flap structure but showed different efficacy profiles in living cells, we conclude that the fluorescence observed from Probe 1-treated cells was solely generated by catalytic activity of the overexpressed intracellular topo I.

2.5. Determination of Cellular DNA Topoisomerase Levels among Various Types of Human Cells using Our Newly Designed Biosensors

As a cancer diagnostic, prognostic, and predictive biomarker, human topoisomerase I is expressed differently between cancerous and healthy cells. To determine whether our newly designed biosensors could serve as effective tools for diagnosing different topo I expression levels between normal and cancer cells, we examined CCD-18Co (colon normal cells) and HT-29 (colon cancer cells) in parallel during our investigations (Fig. 4). As seen in the confocal laser scanning microscopy images (Fig. 4a and 4b), colon normal cells displayed lower fluorescence intensity than HT-29 colon cancer cells. These differences suggest that, unlike colon cancer cells, the amount of topo I in their healthy counterparts was insufficient to generate high fluorescence signals from the probes.

In addition to the aforementioned HT-29 cells, topo I gene-knockdown HT-29 cells were constructed in our lab following previously reported protocols. These two types of cells, native and topo I gene-silenced HT-29, were incubated with Probe 1 in parallel. As shown in Fig. 4c, the fluorescence intensity of gene-knockdown cells was considerably lower than that of native HT-29 cells, indicating insufficient intracellular topo I in the gene-knockdown cells to activate the probes.

Based on the comparison studies between these colon cells, we suggest that our DNA-based probes, as well as our design strategies, could benefit the future development of diagnostic tools for monitoring cellular DNA topoisomerase levels and other biomarkers among various living cells for determining cancer aggressiveness, and for evaluating an individual's predisposition to cancers.

2.6. Examination of the Effects of FDA-Approved Chemotherapeutic Agents on Topoisomerase Enzymatic Activity using Our Newly Designed Biosensors

As a treatment response biomarker, the expression of DNA topoisomerase I often fluctuates in cancer cells before and after anticancer drug treatment. As an FDA-approved anticancer drug and a potent inhibitor of human topo I, irinotecan was used in our study to treat human HT-29 cancer cells (Figure S10). As shown in Figure S10a, HT-29 cells without irinotecan treatment displayed significantly higher fluorescence intensity. However, after irinotecan treatment, the resultant cancer cells showed only a trivial
amount of fluorescence (Figure S10b). These observations are consistent with the proposition that in the
presence of the topo I inhibitor, the catalytic activity of intracellular topo I was drastically suppressed,
which incapacitates catalysis of topo I on the probes inside living cells. In addition, it can be deduced
from these observations that HT-29 colon cancer cells are susceptible to irinotecan treatment because of
the greatly reduced cellular topo I activity.

Besides irinotecan, topotecan, another FDA-approved anticancer drug and inhibitor of topo I,[23, 24] was
examined also during our investigation. Similar to the results obtained from irinotecan studies, cancer
cells before and after topotecan treatment were discernable using Probe 1 as a diagnostic tool (Figure
S10c). Based on our examination of the effects of chemotherapy agents on topo I activity, we propose
that our DNA-based biosensors could be developed as useful clinical tools for monitoring anticancer
treatment responses using a small number of human cells.

**Conclusion**

In this study, we established a new strategy for the effective sensing of DNA topoisomerases and
monitoring their catalytic activities in living cells. In contrast to most conventional molecular probe
designs, our mechanism-based biosensors involve an 89-mer oligonucleotide structure to mimic the
natural substrate of DNA topoisomerases. Taking advantage of the unique mechanism of
topoisomerases, we tricked the enzymes within their catalytic cycles twice to (i) switch on fluorescence
from the oligonucleotides and (ii) resume the original enzymatic process for new rounds of catalysis.
Thus, even though the natural substrates and products of DNA topoisomerases share identical molecular
configurations, these cancer biomarkers can be effectively diagnosed in cell-free systems as well as in
living cells. Considering that human topoisomerases have been generally recognized as key biomarkers
for multiple cancers and recently identified as promising targets for several anticancer drugs, we believe
that our newly designed biosensors could have great potential for development into clinical tools for
determining cancer aggressiveness, monitoring cancer treatment, and evaluating an individual’s
predisposition to developing cancers in the future.

**Experimental Section**

*Preparations of Human Topo I Switch-On Fluorescence Probes:* For synthesis of Probe 1, a solution
containing 10 μM of Oligonucleotide 1, 10 mM Tris-HCl (pH 7.5) and 50 mM NaCl was incubated at 95 °C
for 5 minutes and was further allowed to cool to 25 °C over a time period of 2 hours to let the
oligonucleotide molecules to form self-annealing structures. The same procedures were followed for
preparation of self-annealing Oligonucleotide 2 as well. The two self-annealing structures were mixed in a
molar ratio of 1 : 1 in the presence of 50 mM Tris-HCl (pH 7.5), 5 mM NaCl, 10 mM MgCl2, 1 mM ATP, 10
mM DTT and further incubated at room temperature for 1 hour. To the resultant mixture, 80 units of T4
DNA ligase were added, which was further kept at 16 °C for 2 hours to allow the formation new
phosphodiester bonds between Oligonucleotide 1 and Oligonucleotide 2 (Figure S5). QIAquick nucleotide
removal kit (QIAGEN) was then used for purification of newly formed Probe 1 from its reaction mixtures.
For synthesis of Probe 2 and Probe 3, Probe 2 was prepared through following the same procedures as those for preparing Probe 1 except that Oligonucleotides 2 was replaced with Oligonucleotide 3. In addition, the procedures for preparation of Probe 3 were identical to those for synthesizing Probe 1 as well except that Oligonucleotide 4 was used to replace Oligonucleotide 2.

**Fluorescence Spectroscopic Analysis:** For conducting the studies as shown in Figure 2b and 2d, a solution containing 50 nM of Probe 1, 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 100 mM NaCl, 0.1% BSA, 0.1 mM spermidine, and 5% glycerol was kept at 37 °C for 1 hour in aluminum-foil-protected microcentrifuge tubes in the absence (Figure 2B) or presence of (Figure 2D) 5 nM of human topo I. Fluorescence spectroscopic examinations were carried out at 37 °C in an ultra-micro quartz cuvette (3 x 3 mm light path) using an RF-5301PC fluorescence spectrometer (Shimadzu). Fluorescence emission spectra were measured at an angle of 90° to the 515 nm excitation laser and recorded for every nanometer from 530 to 650 nm. Both excitation and emission slits were set at 5 nm. For conducting the studies as shown in Figure 2f, the same procedures as those for conducting the studies shown in Figure 2d were followed except that different concentrations (0, 0.1, 0.2, 0.5, 1, 2, 3, or 5 nM) of topo I were used. For conducting the studies as shown in Figure 2g, the same procedures as those for conducting the studies shown in Figure 2d were followed except that reaction times were set as 2, 4, 8, 15, 30, 60, 120 minutes respectively. For conducting the studies as shown in Figure 2h, the same procedures as those for conducting the studies shown in Figure 2d were followed except that different enzymes (E. coli topoisomerase I, human topoisomerase IIα, topoisomerase IV, and DNA gyrase) were used. For conducting the studies as shown in Figure 2i, the same procedures as those for conducting the studies shown in Figure 2d were followed except that human topo I was replaced with protein lysates (10 µg) of RC21565-transfected human HEK293T cells and ordinary HEK293T cells respectively.

**Colorimetric Imaging under Ultraviolet Illumination:** A solution containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 100 mM NaCl, 0.1% BSA, 0.1 mM spermidine, and 5% glycerol in the presence of 5 nM of topo I (Figure 2e) and 0 nM of topo I (Figure 2c) was incubated at 37 °C for 1 hour. The reactions were stopped by heating the samples at 65 °C for 15 min. Upon their condensation under vacuum, the resultant samples were transferred to quartz cuvettes (Hellma 100-1-20 QS). Photographs of reaction mixtures in quartz cuvettes were obtained by using a Sony A7II digital camera under ultraviolet illumination (302 nm) in a dark room.

**Cell Culture:** HT-29 human colon cancer cells and siRNA-treated HT-29 cells were cultivated in McCoy’s 5A (modified) medium supplemented with 10% (v/v) fetal bovine serum. CCD-18Co non-tumorigenic human colon cells were cultured in Eagle’s Minimum Essential Medium (EMEM) supplemented with 10% (v/v) fetal bovine serum. All the cells were cultured at 37 °C in a Forma 371 Steri Cycle incubator (Thermo Fisher Scientific) under a humidified atmosphere of 5% carbon dioxide. Replacement of growth medium was carried out every two to three days during the cultivation periods. After reaching 80–90% confluency, the cells were trypsinized with 0.25% trypsin-EDTA and subcultured at a ratio of 1 : 4.
**Preparation of Topo I Gene-Knockdown HT-29 Cells:** Topo I gene-knockdown HT-29 cells were obtained by transfection of chemically synthesized small interfering RNA (ON-TARGETplus Human TOP1 siRNA) following the manufacturing protocols. Briefly, stock solutions of Lipofectamine RNAiMAX and siRNA were mixed in separate microcentrifuge tubes using serum-free media as solvents at room temperature using a volume ratio of 1:1. The resultant RNA/lipid mixture was further incubated at room temperature for 5 minutes. The resultant mixture was then incubated with HT-29 cells at room temperature for 48 hours.

**Confocal Laser Scanning Microscopic Studies:** For conducting the studies as shown in Figure 3b and 3d, cultured cells were seeded in 35-mm imaging dishes (Ibidi µ-Dishes) and further allowed to grow at 37 °C in the presence of 5% carbon dioxide to yield a final cell density of \(5 \times 10^4\) cells/cm\(^2\). Probe 1 (200 nM) were pre-mixed with Lipofectamine LTX and PLUS Reagent according to the manufacturing protocol followed by incubating them with cultured HT-29 cells at 37 °C for 36 hours. These samples were then gently removed and the remaining cells were washed twice with phosphate-buffered saline (pH 7.4). When it was necessary, two drops of NucBlue Live ReadyProbes reagent were incubated with the acquired cells for extra 10 minutes for the nuclear staining purpose. Confocal laser scanning microscopy was carried out on an LSM 800 confocal microscope (Zeiss) coupled with 40× or 100× oil immersion objectives. Relative fluorescence intensity per cell was quantified by the mean grey value of microscopy images with the areas manually selected (n = 120 cells) using an open-source software ImageJ. For conducting the studies reported in Figure 3c, the same procedures as those for conducting the studies as shown in Figure 3b were followed except that Probe 1 was replaced with Probe 2. For conducting the studies reported in Figure 3e, the same procedures as those for conducting the studies as shown in Figure 3b were followed except that HT-29 cells were replaced with CCD-18Co cells. For conducting the studies reported in Figure 3f, the same procedures as those for conducting the studies as shown in Figure 3b were followed except that HT-29 cells were replaced with topo I gene knockdown HT-29 cells.

**Declarations**

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**Authors’ contributions**

HZ and TL designed the research. SB, GG, and HZ conducted the experiments, statistical analysis, and data interpretation. SB, HZ, and TL contributed to writing and assisted in editing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

All data and materials are available within the manuscript and in additional files.

Ethics approval and consent to participate

Not applicable.

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Competing interests

The authors declare no conflict of interests in the paper.

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Figures
Figure 1

Schematic illustrations of topoisomerase I-catalyzed reactions on duplex DNA. (a) An example of a well-studied topo I-binding DNA sequence (Duplex 1).[28] (b) The catalytic intermediate of topo I as reported by the Westergaard group.[28] In the reaction, topo I generates a nick site on Duplex 1 and reseals the nick site unceasingly. (c) A structural illustration of one of our newly designed DNA-based biosensors (Probe 1). (d) An illustration of the Probe 1 isoform that is in dynamic equilibrium with Probe 1. This equilibrium...
will shift to favor the formation of Probe 1 when Probe 1 is consumed by the action of topo I. (e) The catalytic mechanism of human topo I on Probe 1. Steps 1-3: Topo I is misled to act on the oligonucleotide, trigger the release of Fragment 1, and consequently switch on the quenched fluorescence. Steps 4-6: Topo I is tricked into accepting the flapped trinucleotide as part of its original substrate for proceeding with the subsequent religation process. This action allows topo I to resume its original structure for new rounds of catalysis.

**Figure 2**
Examination of the sensitivities of the newly designed biosensors to human topo I in cell-free systems. (a) Illustration of topo I-activated restoration of fluorescence from Probe 1. (b) The fluorescence spectrum of Probe 1 in the absence of topo I. (c) Colorimetric study of Probe 1 under UV irradiation in the absence of topo I. (d) The fluorescence spectrum of Probe 1 in the presence of topo I. (e) Colorimetric study of Probe 1 under UV irradiation in the presence of topo I. (f) The fluorescence spectra of Probe 1 in the presence of different concentrations of topo I. (g) Correlation of fluorescence intensities with the incubation times of Probe 1 and topo I. (h) Examination of the specificity of Probe 1 for other non-target topoisomerases. (i) Examination of the performance of Probe 1 in protein lysates of topo I-overexpressed cells and ordinary cells. Unless otherwise stated, all reactions were performed with 50 nM Probe 1 and 5 nM human topo I at 37 °C for 1 hour in aluminum-foil-protected tubes. Detailed experimental procedures are described in Supporting Information. Data shown in G, H, and I are expressed as the mean ± standard deviation (*P < 0.005; n = 5).
Figure 3

Examination of the responsiveness of the DNA-based biosensors in human cancer cells. (a) Schematic illustration of Probe 1 for sensing of human topo I in living cells. (b) Confocal laser scanning microscopy images of Probe 1-treated cancer cells. The high fluorescence intensity in Cy3 channel (b1) indicates the successful restoration of fluorescence from Probe 1 by catalytic actions of intracellular topo I. (c) Schematic illustration of actions of Probe 2 in living cells. (d) Confocal laser scanning microscopy images of Probe 2-treated cancer cells. The low fluorescence intensity in the Cy3 channel (d1) indicates
that without the critical 5’ flap structure, Probe 2 did not generate fluorescence even in the presence of intracellular topo I. Cells were incubated with 200 nM probes at 37 °C for 36 h prior to confocal examination. Nuclei were stained with Hoechst 33342.

Figure 4

Determination and comparison of cellular DNA topoisomerase levels among HT-29 colon cancer cells (a), CCD-18Co colon normal cells (b), and topo I gene-knockdown HT-29 cells (c) using our newly designed biosensors. Cells were incubated with 200 nM probes at 37 °C for 36 h prior to confocal examination. Nuclei were stained with Hoechst 33342. Relative fluorescence intensity per cell was quantified from microscopic images with the areas manually selected (n = 120 cells) using ImageJ. The observed high fluorescence intensity in a1 is affiliated with actions of highly abundant topo I in colon cancer cells HT-29, while the observed low fluorescence intensities in b1 and c1 are affiliated with insufficient amounts of topo I in colon normal cells and topo I gene-knockdown cells to activate Probe 1.

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