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Communication

Quantum Dots Based DNA Nanosensors for Amplification-Free Detection of Human Topoisomerase I

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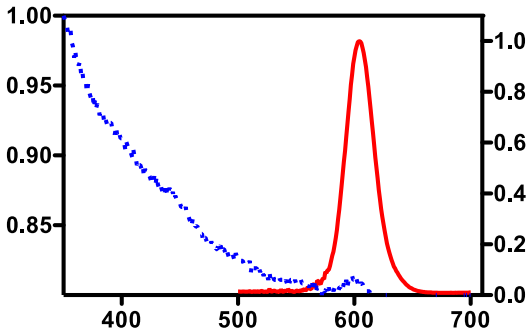
Supplementary Information

1. Preparation of DNA Nanosensors:

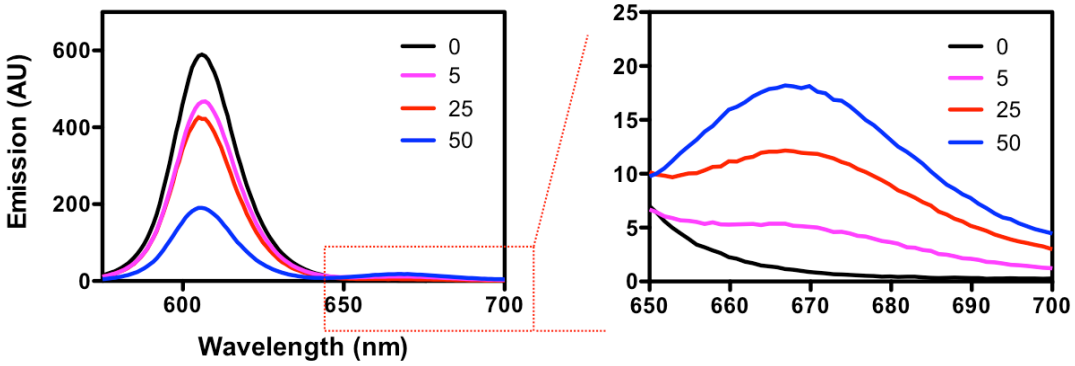
Oligonucleotides, listed in Table 1, were synthesized and HPLC purified by DNA-technology, Denmark. Streptavidin-functionalized CdSe-ZnS QDs conjugates (Qdot® 605 ITK™ Streptavidin Conjugate) were purchased from Invitrogen (Hayward, CA). The absorption and emission spectra of QDs (Lot# 1084419), as shown in Supplementary Figure 1, were pre-evaluated prior the experiments. The DNA nanosensors consisting of two double stranded DNA strands, cleavage/cleavage-complementary (CL) and the ligation/ligation-complementary (LI) strands, were hybridized in 1X TE buffer (10mM Tris-HCl and 1mM EDTA at pH 7.5) following standard protocol. The ligation detector was assembled by conjugating the ligation strand (ligation/ligation-complementary) onto QDs, through biotin-streptavidin interaction. The molar ratio of LI strand to QD was kept in a ratio of LI : QD = 50 : 1. The ratio was empirically determined at where the maximum FRET efficiency occurred, as shown in Supplementary Figure 2.

Supplementary Table 1: Sequences of the Designed DNA Nanosensors

Cleavage	5'-Cy5-TGA CCG AAA AGA CTT ^ AGA AAA ATT TTT-3' (^ cleavage site)
Cleavage-complementary	5'-CTA AGT CTT TTC GGT CA-3'
Ligation	5'-AGA AAA ATT TTT GGC CG-Biotin-3'
Ligation-complementary	5'-CGG CCA AAA ATT TTT-3'



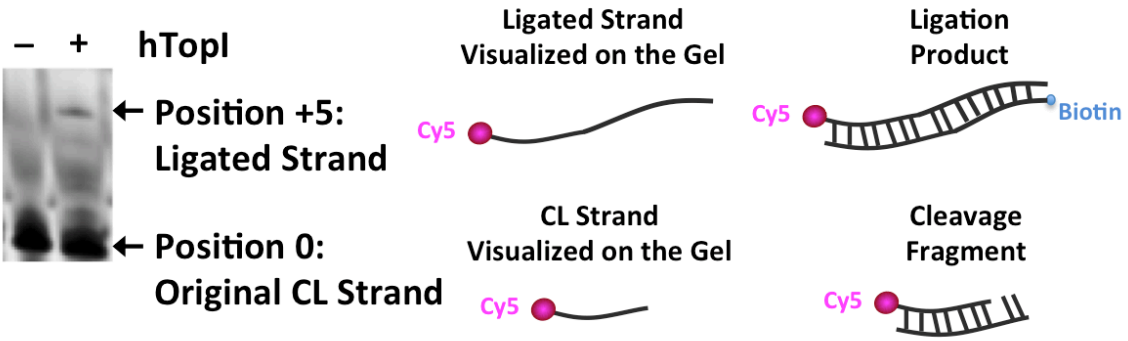
Supplementary Figure 1. Fluorescence Spectra of the QDs: Absorption and emission spectra of the used QDs were pre-evaluated prior to experiments. The peak emission was observed at 608nm, which fit with datasheet supplied by the vendor.



Supplementary Figure 2. Maximum FRET: Ratio of LI strand to QD, which would in turn determine the maximum amount of Cy5 acceptor versus QD donor, was previously determined by a control experiment. Double-stranded DNA simulated the cleavage-ligation product, dual-labeled with biotin and Cy5, was mixed with QDs in various ratios. The FRET efficiency was observed saturated at the ratio of 50. Further increase of the ratio did not observed significant increase of FRET (data not shown).

2. Cleavage-ligation of hTopI:

To validate whether the designed strand enabled proper hTopI cleavage and ligation, the samples were analyzed by gel electrophoresis as described previously[1]. Briefly, 12% denaturing polyacrylamide gel was made of 15 ml 40% acrylamide, 10 ml H_2O and 5 ml 10x TBE (890 mM Tris-HCl, 890 mM boric acid, and 10mM EDTA) , and 22.5g urea. Additionally, TEMED and 10% APS was added to cure the gel. Gel scanning was conducted using a Typhoon scanner (GE Healthcare Biosciences, Uppsala) with a setting for the observation of Cy5 (Excitation: 633nm, Emission: 670nm). The gel image, shown in Supplementary Figure 1, was processed using Photoshop (CS5, Adobe Systems, San Jose, California, USA).



Supplementary Figure 2. Functionality of hTopI on the Designed DNA-nanosensor: Gel electrophoresis was used to investigate whether hTopI could properly cleave and religate the designed DNA nanosensors. The band positions for the original CL strand and religated strands, were estimated from the DNAs sequences. Cy5 modification of the strands allowed a direct visualization of the gel with post-staining. Clearly seen from the band expected for the sample containing hTopI, which produced the ligated strand positioned at +5, ligation of the LI DNA fragment occurred in the presence of hTopI.

3. Yeast Strains and Construction of hTopI Expression Plasmids/ Expression and Purification of hTopI and Preparation of Yeast Extracts

The yeast *Saccharomyces cerevisiae* TopI-null strain RS190 was a kind gift from R. Sternglanz (State University of New York, Stony Brook, NY, USA). The plasmid pHT143 was transformed into the *S. cerevisiae* strain RS190 to generate RS190+hTopI for expression of recombinant full-length hTopI, as described previously[1]. The yeast cells were grown, and hTopI expression was induced by in 2% galactose for 16 hours as described in[2]. Yeast crude extracts were prepared by resuspending yeast cells in 500 mM elution buffer (10% Glycerol, 0.5M NaCl, DTT (1:1000) and PMSF (1:2000)) followed by destroying the cells through vortexing the cells vigorously with glass beads (Ø 425-600 μm, acid-washed, G8772-1KG, Sigma-Aldrich) for 20 minutes 3 times. After the destruction of the cells the cell debris was spun down by centrifugation and the supernatant was collected as crude yeast extracts. The extracts were then used directly for the QFRET assay. For the experiments with purified enzyme, purification of hTopI was performed following previously established protocols[3].

4. Cell Culture and Preparation of Nuclear Extracts

Caco-2 cells (Homo sapiens Colon Colorectal adenoca) were cultured in complete medium (Minimum Essential Media, MEM-GlutaMAX, supplemented with Non-Essential Amino Acid, 20% fetal bovine serum (FBS), 1% penicillin, and 1% streptomycin). HT-29 (derived from colon-carcinoma microtissue) were cultured in McCoy's 5A modified medium supplemented with 10% FBS, 1% penicillin, and 1% streptomycin. Cells were harvested with 0.5% Trypsin-EDTA (Gibco) and media was then discarded. Cells were subsequently washed in phosphate-buffered saline (PBS) prior to nuclear extraction. Extraction was executed following the protocol established previously[4]. Briefly, approximated $1x10^6$ cells/mL of cells were lysed in 1 mL of lysis buffer (0.1% NP-40, 10 mM Tris, pH 7.9, 10 mM $MgCl_2$, 15 mM NaCl, 0.1 mM phenylmethyl sulfonyl fluoride). After cell lysis the nuclei were extracted in 80 μL of extraction buffer (0.5 M NaCl, 20 mM HEPES, pH 7.9, 20% glycerol, 0.1 mM phenylmethyl sulfonyl fluoride). The extracts were used directly for the QFRET assay.

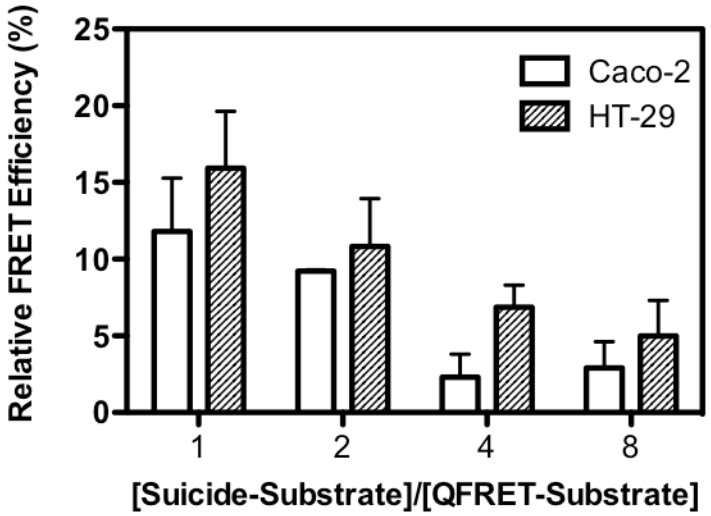
5. Specificity Validated by Competition Assay

Specificity of the designed DNA nanosensors was evaluated through a competition assay with a standard suicide substrate, that acts as a mechanism based inactivator of hTopI by allowing cleavage and covalent attachment of the enzyme but preventing the subsequent ligation step that releases and reactivates the enzyme as described in[5]. Hence, the suicide substrate (38SC/47NCL, sequences listed in Supplementary Table 2), served as a competing substrate to the DNA nanosensor by depleting the extract for active hTopI prior to the addition of the QFRET biosensor system. Prior to the competition assay, the suicide substrate was phosphorylated at the 5' end to prevent ligation of this end leading to release and re-activation of the enzyme. Briefly, 100 pmol of suicide substrate was mixed with 1 μl of T4 Polynucleotide kinase (New England Biolabs, Ipswich, MA, USA) in the buffer containing 1x polynucleotide kinase (PNK) buffer and 1mM Adenosine triphosphate (ATP). The reaction was then incubated at 37°C for 30 min.

Supplementary Table 2: Sequences of the Suicide Substrate used for Competition Assay

47NCL	5'-AA AAA TTT TTC TAA GTC TTT TAG ATC CTC TAG AGT CGA CCT ^ GCA GGC-3' (^ hTopI binding site)
38SC	5'-GC CTG CAG GTC GAC TCT AGA GGA TCT AAA AGA CTT AGA-3'

The competition assay was conducted by pre-incubating fixed amount of cell extracts with varying amount of suicide substrates at 37°C for 5 min, prior to the addition of the designed QFRET DNA nanosensor and proceeded to the normal assay protocol. As shown in Supplementary Figure 2, the detected QFRET signal dropped as the amount of competitive suicide substrates increased, suggesting that the DNA nanosensor was specific to hTopI.



Supplementary Figure 3. Competition Assay: A set of suicide substrate was used to probe the specificity of the designed DNA nanosensors. In the presence of suicide substrate, hTopI covalently bound to the suicide substrate and no longer being detectable by the QFRET substrate. As expected, the detected QFRET signal dropped when the suicide substrate was in excess compared to QFRET substrate.

5. QFRET Assay and Analysis of hTopI Activity

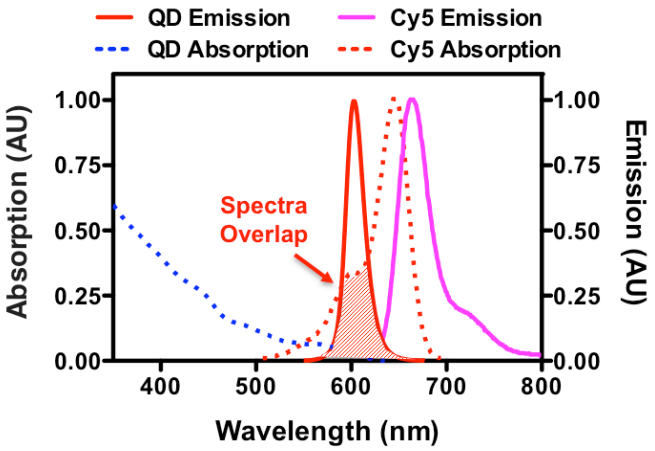
hTopI activity was measured by its cleavage and re-ligation. Titrated amount of purified hTopI, yeast extract, or cell extract was incubated with 2.5 pmol of CL strand in 1X TopI buffer (1 mM Tris-HCl, 5 mM $CaCl_2$, and 5 mM $MgCl_2$, pH 7.5) for 30min at 37°C. Subsequently, the hTopI-CL complex was then mixed with the ligation detector (preconjugated with 2.5 pmol of CL strand and 0.1pmol of QD) in 1X TopI buffer for 30min at 37°C. The mixture was used for investigation without further treatment.

Fluorescence emission spectra were obtained by a spectrofluorometer (Cary Eclipse, Varian, now Agilent Technologies, Santa Clara CA). High throughput fluorescence measurement was achieved by a commercially available fluorescent microplate reader

(Fluostar, BMG Labtech, Durham, NC). The emissions for QD605 (bandpass 570-590 nm) and FRET-mediated Cy5 (660-680 nm) were measured upon excitation at 390 nm.

The Förster radius R_0, defined as where 50% of energy transfer occurs, was calculated to be 65.09 Å for the selected pair of QD605 and Cy5, according to Equation 1. Parameters used in the estimation included, the refractive index of the medium (n=1.4), the unperturbed donor photoluminescence quantum yield (Q_D=0.4), the spectral integral from the overlap of donor emission and acceptor absorption ($J(\lambda)=1.24\times10^{16}$ $M^{-1}cm^{-1}nm^4$, see also Supplementary Figure 4) and the relative orientation of the donor and acceptor dipoles ($\kappa^2 \sim 2/3$)[6].

$$R_0 = 0.211[\kappa^2 n^{-4} Q_D J(\lambda)]^{1/6} \quad \text{(in Å)} \qquad \text{Equation (1)}$$



Supplementary Figure 4. Selection of QFRET Pair: The QFRET pair was selected for sufficient spectra overlap, rendering efficient energy transfer within the distance between donor and acceptor designed in this study, 10.56nm (32 base pairs).

Empirically, FRET was characterized using the ratiometric FRET[7, 8], the relative FRET efficiency E_{Rel} was calculated according to Equations (1), after subtraction of background fluorescence.

$$E_{Rel} = \frac{I_{FRET}}{I_{FRET} + I_{QD605}} \qquad (1)$$

At least four individual experiments were conducted for each condition. Error bars were calculated as the standard deviation from each independent experiment. The statistical significance was determined using an one-way ANOVA test (Prism 5.0, GraphPad Software, La Jolla, CA).

References:

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