Supporting Information

An amplified single-walled carbon nanotube mediated chemiluminescence turn-on sensing platform for ultrasensitive DNA detection

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Experimental Section

Materials: All oligonucleotides were purchased from the Sangon Biotech Co. (Shanghai, China) and purified by HPLC. The sequences of the involved oligonucleotides were given in Table S1. The exonuclease III (Exo III) was purchased from New England Biolabs. The carboxylated single-walled carbon nanotubes (SWNTs) (purity >90%) were purchased from Aldrich (St. Louis, MO). All other chemicals were of analytical grade. The water was purified by employing a Milli-Q plus 185 equip from Millipore (Bedford, MA, USA), and used throughout the work. All oligonucleotides were dissolved in 20 mM Tris-HCl solution (pH 7.4) containing 50 mM NaCl and 10 mM $MgCl_2$. Reaction buffer for CRET assay is 20 mM Tris-HCl solution (pH 7.4) containing 50 mM NaCl, 10 mM $MgCl_2$ and 10 μM HRP. The oxidizer solution was 40 mM Na_2CO_3 buffer (pH 11.0) containing 30 mM H_2O_2.

Table S1. Sequences of DNA used in this work

Strand	Sequence
DNA-1	5'-CAGAGGCAGTAACCAGTACTGAT-ABEI-3'
DNA-2	5'-ATCAGTACTGGTTTACTGCCTCTGATCTGA-3'
DNA-3	5'-ATCAGTACTG**A**TTTACTGCCTCTGATCTGA-3'
DNA-4	5'-TTGGGAAAGAATGGGGCCCAGTGTGTGGGC-3'
DNA-5	5'-TTATTATGGAAGACGGCCCTTCT-ABEI-3'
DNA-6	5'-GCCCTTGTAAGGTACCGTCTTCCCCAGAAGGGCCGATGATT-3'
DNA-7	5'-GGGAAGACGGTACCTTACTCAACTT-3'

Instrumentation: Chemiluminescence measurements were performed on a LS-55 spectrofluorometer (Perkin-Elmer, USA). UV-visible spectra were measured with a TU-1901 UV-visible spectrophotometer (Beijing Purkinje General Instrument Co., Ltd. China). Fluorescence polarization measurements were performed on an FL3-P-TCSPC system (Jobin Yvon, Inc., Edison, NJ, USA). Fluorescence polarization of the sample solution was monitored by exciting the sample at 370 nm and measuring the emission at 450 nm. And slits for both the excitation and the emission were set at 5 nm.

Preparation of ABEI-DNA/SWNTs probe: Forty five microgramme of the carboxylated SWNTs was added to a 1 mL Tris-HCl ABEI labeled DNA (ABEI-DNA-1, or ABEI-DNA-5, each 50 nM) and was allowed to incubate for 1 h at room temperature. The resulting solution was used directly for CRET assay.

Analysis of the target DNA using the ABEI-DNA-1/SWNTs hybrid: Various concentrations of the target DNA-2 was added to a 800 μL of ABEI-DNA-1/SWNTs solution (in reaction buffer) containing 40 units of Exo III in a quartz cuvette, and the mixture was incubated at 37 ^{o}C for 2 h. Then, 500 μL of oxidizer solution was immediately poured into the quartz cuvette, and CL spectrum was recorded immediately with a LS-55 luminescence spectrometer.

Analysis of the target DNA using the ABEI-DNA-5/SWNTs hybrid and hairpin DNA-6: The target DNA-7 at different concentrations was added to a 800 μL of ABEI-DNA-9/SWNTs solution (in reaction buffer) containing DNA-6 (50 nM) and Exo III (40 units) in a quartz cuvette, and the mixture was incubated at 37 ^{o}C for 2.5 h. Then, 500 μL of oxidizer solution was immediately poured into the quartz cuvette, and CL spectrum was recorded immediately with a LS-55 luminescence spectrometer.

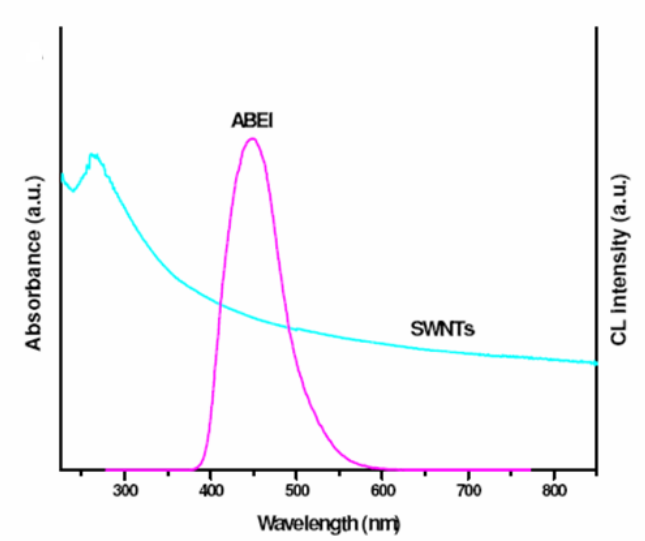


Figure S1. UV/Vis spectrum of SWMTs, spectrally overlapping with the CL spectrum of ABEI.

Result of control experiment using a free ABEI solution simply mixed with SWNTs:

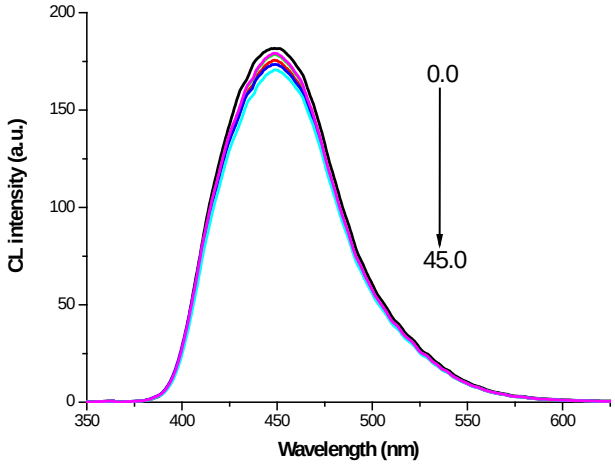


Figure S2. CL titration profile of non-conjugated ABEI by SWNTs with concentrations ranging from 0 to 45 μg/mL at intervals of 9 μg/mL.

Result of the ABEI-DNA-1/SWNTS hybrid with Exo III signal amplification upon the analysis of the target DNA-2 at different reaction time:

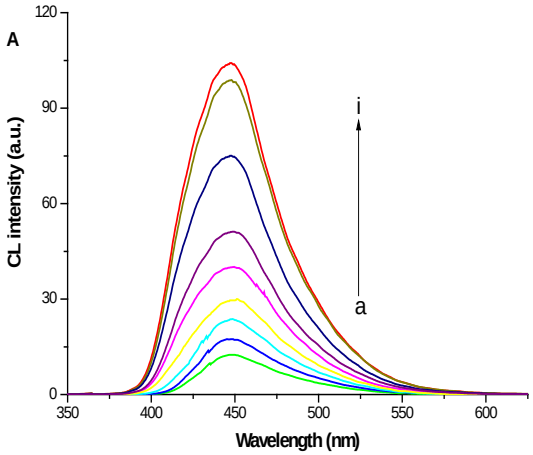


Figure S3. Time-dependent CL spectra of the ABEI-DNA-1/SWNTs hybrid with Exo III signal amplification: curve (a), in the absence of the target DNA-2; curves (b-i) after introducing 50 pM DNA-2. CL signal was monitored from 0 to 120 min at a time interval of 15 min. The assay was performed in a 20 mM Tris-HCl solution (pH 7.4), containing the ABEI-DNA-1/SWNTs hybrid (50 nM DNA-1, 45 μg/mL AWNTs), Exo III (40 units), 50 mM NaCl and 5 mM $MgCl_2$.

Result of control experiments for the ABEI-DNA-1/SWNTS hybrid upon the analysis of the target DNA-2:

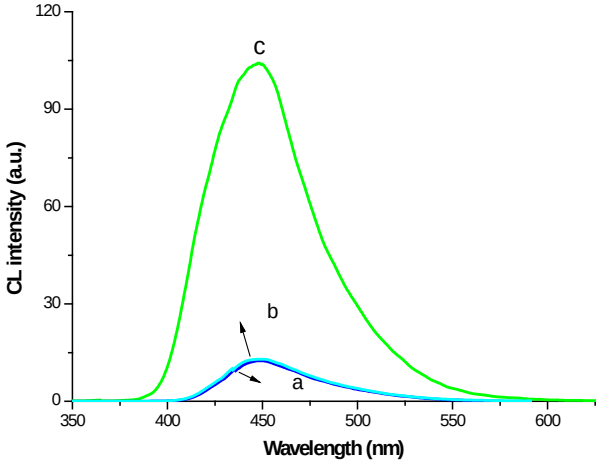


Figure S4. CL spectra for the ABEI-DNA-5/SWNTS hybrid upon the analysis of the target DNA-2 (50 pM). Curve a is obtained from only the ABEI-DNA-1/SWNTS hybrid; curve b is is obtained from the ABEI-DNA-1/SWNTS and DNA-2; curve c is obtained from the ABEI-DNA-1/SWNTS, DNA-2 and Exo III. The reaction time is 120 min.

Characterization of DNA-SWNTs interactions in Scheme 1:

As fluorescence polarization measurements are commonly used to probe molecular interactions,[1] the fluorescence polarization value *P* of ABEI-DNA-1 and the ABEI-DNA-1/SWNTs complex before and after hybridization with DNA-2 (20 nM) was measured for evidence of the adsorption of single-stranded DNA on SWNTs and the release of ABEI-DNA-1/DNA-2 duplex from SWNTs. The results are shown in Figure S5. In the absence of SWNTs, the *P* value of free ABEI-DNA-1 was 35, whereas that for the ABEI-DNA-1/SWNTs complex was 363, indicating that the single-stranded DNA is adsorbed on SWNTs. However, upon the addition of DNA-2 (20 nM) into a mixture of the ABEI-DNA-1/SWNTs complex, the ABEI-DNA-1/DNA-2 duplex was formed, the *P* value decreased to be 214. These results primarily indicated that the formation of the ABEI-DNA-1/DNA-2 duplex resulted in the detachment of ABEI-DNA-1 from SWNTs. The above results were further confirmed by microchip electrophoresis (MCE) with chemiluminescence (CL) detection, which was perform on a douple T microchip as the procedure described in our previous work.[2] The results are shown in Figure S6. Trace a was obtained from a solution containing ABEI-DNA-1 only. Trace b was obtained from a solution containing ABEI-DNA-1 and DNA-2. A new peak at a longer migration time that was from the ABEI-DNA-1/DNA-2 duplex was observed. Trace c was obtained from the supernatant of a reaction solution containing ABEI-DNA-1 and SWNTs. Compared with trace a, the peak from ABEI-DNA-1 was disappeared, which indicated that the single-stranded DNA is adsorbed on SWNTs. Trace d was obtained from the supernatant of a reaction solution containing ABEI-DNA-1, DNA-2 and SWNTs. Compared with trace c, a new peak was observed. The migration time of this peak was consistent with the peak of the ABEI-DNA-1/DNA-2 duplex in trace b. It was from the ABEI-DNA-1/DNA-2 duplex. This result demonstrated that the formed ABEI-DNA-1/DNA-2 duplex could be released from SWNTs. Furthermore, these results were also consistent with the CRET-based assays as shown in the text.

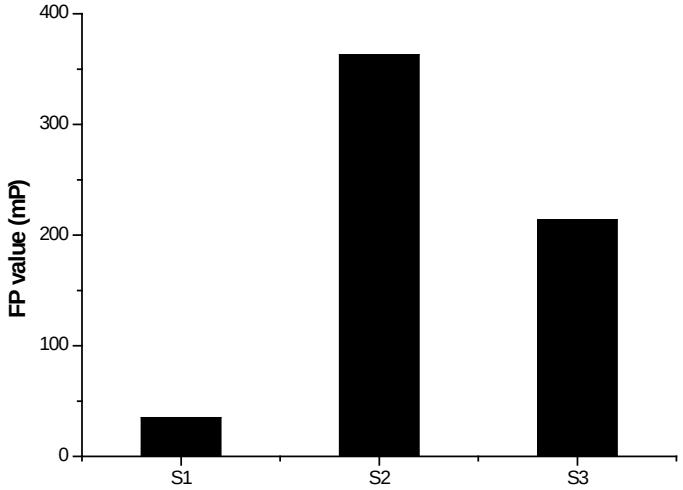


Figure S5. Changes of fluorescence polarization values of (S1) ABEI-DNA-1 (50 nM), (S2) ABEI-DNA-1 (50 nM) + SWNTs (45 μg/mL), (S3) ABEI-DNA-1 (50 nM) + SWNTs (45 μg/mL) + DNA-2 (20 nM).

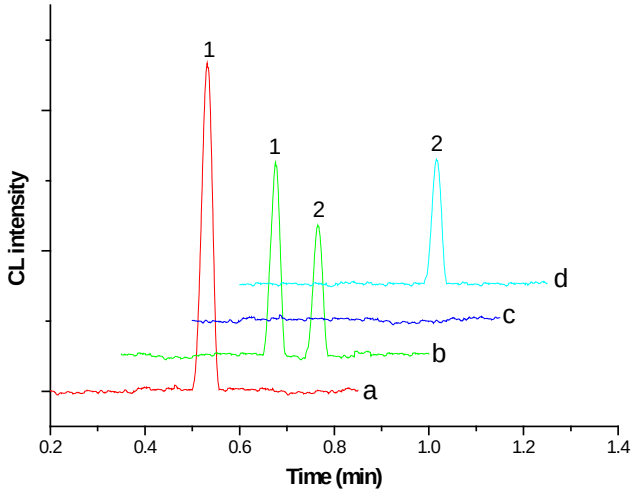


Figure S6. Electropherograms obtained from the analysis of different samples: (a) ABEI-DNA-1 (50 nM), (b) ABEI-DNA-1 (50 nM) and DNA-2 (20 nM), (c) the supernatant of a reaction solution containing ABEI-DNA-1 (50 nM) and SWNTs (45 μg/mL), (d) the supernatant of a reaction solution containing ABEI-DNA-1 (50 nM), DNA-2 (20 nM) and SWNTs (45 μg/mL). All sample solutions were incubated for 100 min and then centrifuged at 16,000 rpm for 30 min before analysis. MCE conditions: 20 mM Tris-HCl running buffer (pH 8.0) containing 10 μM HRP; the oxidizer solution was 20 mM Na_2CO_3 buffer (pH 11.0) containing 110 mM H_2O_2. Voltages used in MCE-CL detection were as described in Ref. 2 in the Supporting Information. Peaks: 1. ABEI-DNA-1; 2. the ABEI-DNA-1/DNA-2 duplex.

Exonuclease III cleavage assay in Scheme 1 by microchip electrophoresis:

The samples for MCE assays were obtained as the following: The Exo III (40 units) were added into a mixture (1 mL) of the ABEI-DNA-1/SWNTs complex and DNA-2 (50 pM) in assay buffer, and the mixture was incubated for various times (30 min, 60 min, 90 min, and 120 min) at 37 ^{o}C. The mixtures were centrifuged at 16,000 rpm for 30 min. The supernatant was used for MCE assay. A blank sample was prepared similar to the procedure mentioned above except no addition of Exo III. MCE assays with CL detection were perform on a douple T microchip as the procedure described in our previous work.[2] The MCE assay results are shown in Figure S6. As can be seen, a new peak obtained from the cleaved products was observed in the electropherogram obtained from the reaction solution (ABEI-DNA-1/SWNTs and DNA-2) with Exo III compared with that obtained from the solution without Exo III (Figure S7A). Moreover, as the reaction time was prolonged, the peak height (CL intensity) of this peak gradually increased (Figure S7B). These results indicated that the ABEI-DNA-1 could be cleaved in the presence of Exo III and DNA-2, and the cleaved products could be released from SWNTs.

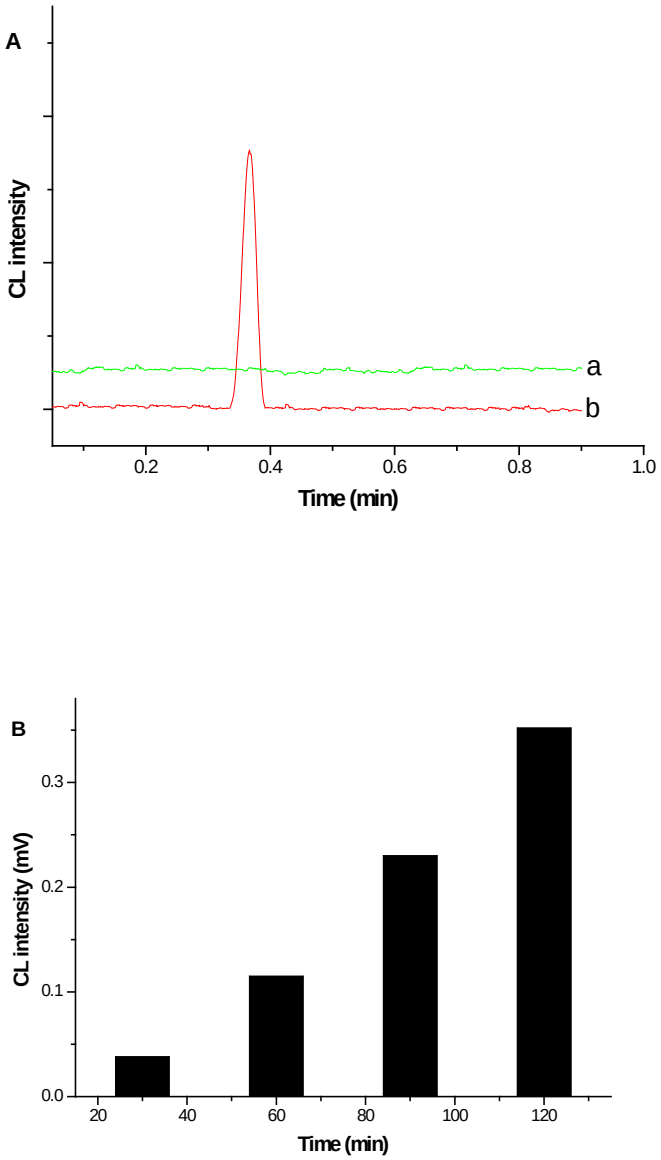


Figure S7. (A) Electropherograms obtained from the analysis of (a) the supernatant of a reaction solution containing ABEI-DNA-5/SWNTs (50 nM DNA, 45 μg/mL) + DNA-6 (50 pM) and (b) the supernatant of a reaction solution containing ABEI-DNA-5/SWNTs (50 nM DNA, 45 μg/mL) + DNA-6 (50 pM) + Exo III (40 units). All sample solutions were incubated for 120 min and then centrifuged at 16,000 rpm for 30 min before analysis. MCE conditions: 20 mM Tris-HCl running buffer (pH 8.0) containing 10 μM HRP; the oxidizer solution was 20 mM Na_2CO_3 buffer (pH 11.0) containing 110 mM H_2O_2. Voltages used in MCE-CL detection were as described in Ref. 2 in the Supporting Information. (B) CL intensity of the cleaved products was obtained from different reaction time.

Time-dependent CL spectra of the ABEI-DNA-5/SWNTs hybrid:

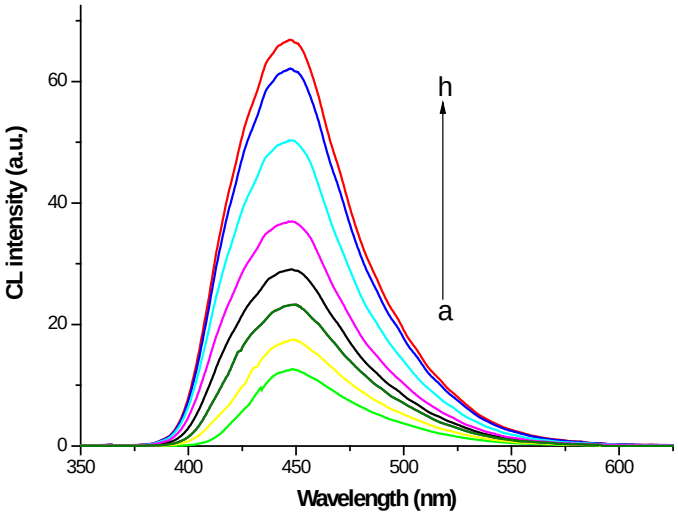


Figure S8. (A) Time-dependent CL spectra of the ABEI-DNA-5/SWNTs hybrid: curve (a), in the absence of the target DNA-7; curves (b-h) upon interaction with 50 pM DNA-7. CL assays were tested from 0 to 140 min at time intervals of 20 min. The assay was performed in a 20 mM Tris-HCl solution (pH 7.4), containing the ABEI-DNA-5/SWNTs hybrid (50 nM DNA-5, 45 μg/mL SWNTs), Exo III (40 units), 50 nM DNA-6, 50 mM NaCl and 5 mM $MgCl_2$.

Result of control experiments for the analysis of target DNA-7 using the ABEI-DNA-5/SWNTs hybrid and hairpin DNA-6:

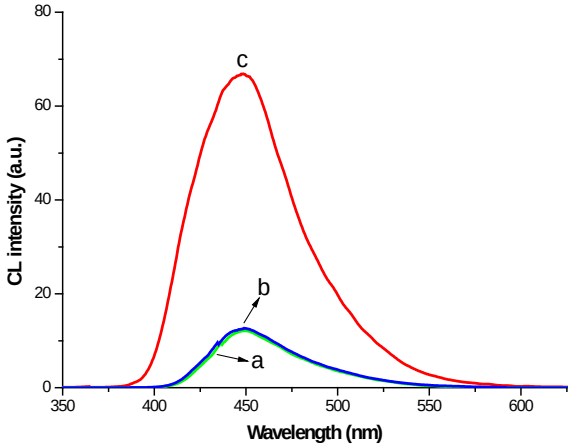
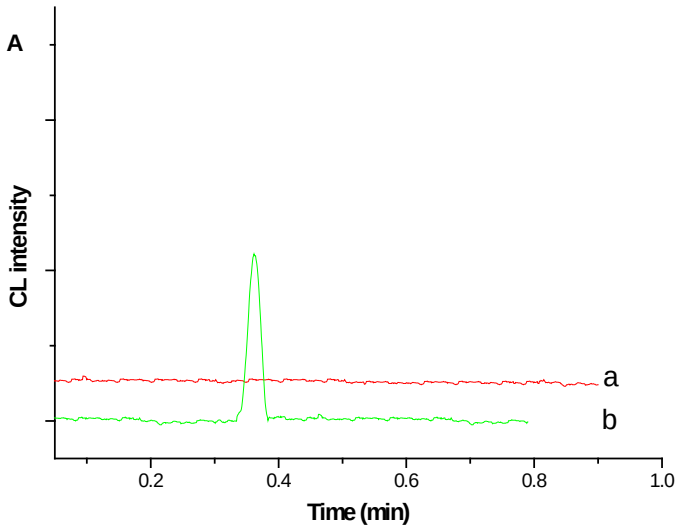


Figure S9. CL spectra for the ABEI-DNA-5/SWNTS hybrid upon the analysis of the target DNA-7 (50 pM) by using DNA-6. Curve a is obtained from only the ABEI-DNA-5/SWNTS hybrid and DNA-7; curve b is is obtained from the ABEI-DNA-5/SWNTS, DNA-6 and DNA-7; curve c is obtained from the ABEI-DNA-5/SWNTS, DNA-6, DNA-7 and Exo III. The reaction time is 140 min.

Exonuclease III cleavage assay in Scheme 2 by microchip electrophoresis:

The samples for MCE assays were obtained as the following: The Exo III (40 units) were added into a mixture (1 mL) of the ABEI-DNA-5/SWNTs complex and DNA-7 (50 pM) in assay buffer, and the mixture was incubated for various times (30 min, 60 min, 90 min, 120 min, and 140 min) at 37 ^{o}C. The mixture was centrifuged at 16,000 rpm for 30 min. The supernatant was used for MCE assay. A blank sample was prepared similar to the procedure mentioned above except no addition of Exo III. MCE assays with CL detection were perform on a douple T microchip as the procedure described above. The MCE assay results are shown in Figure S10. As shown in Figure S10A, a new peak obtained from the cleaved products was observed in the electropherogram obtained from the reaction solution (ABEI-DNA-5/SWNTs and DNA-7) with Exo III compared with that obtained from the solution without Exo III. Moreover, as the reaction time was prolonged, the peak height (CL intensity) of this peak gradually increased (Figure S10B). These results indicated that the ABEI-DNA-5 could be cleaved in the presence of Exo III and DNA-7, and the cleaved products could be released from SWNTs.



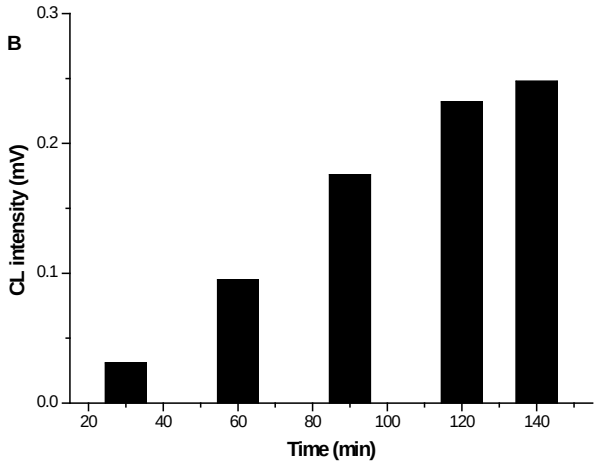


Figure S10. (A) Electropherograms obtained from the analysis of (a) the supernatant of a reaction solution containing ABEI-DNA-5/SWNTs (50 nM DNA, 45 μg/mL) + DNA-7 (50 pM) and (b) the supernatant of a reaction solution containing ABEI-DNA-5/SWNTs (50 nM DNA, 45 μg/mL) + DNA-7 (50 pM) + Exo III (40 units). All sample solutions were incubated for 140 min and then centrifuged at 16,000 rpm for 30 min before analysis. MCE conditions: 20 mM Tris-HCl running buffer (pH 8.0) containing 10 μM HRP; the oxidizer solution was 20 mM Na_2CO_3 buffer (pH 11.0) containing 110 mM H_2O_2. Voltages used in MCE-CL detection were as described in Ref. 2 in the Supporting Information. (B) CL intensity of the cleaved products was obtained from different reaction time.

References

[1] New Trends in Fluorescence Spectroscopy. Applications to Chemical and Life Sciences (Eds.: B. Valeur, J. C. Brochon), Springer, Berlin, 2001.

[2] S. Zhao, Y. Huang, M. Shi, Y. M. Liu, *J. Chromatogr. A*, 2009, **1216**, 5155.