Supplementary Information

A Label-free and Turn-on Fluorescence Strategy for DNA Detection with a Wide Detection Range Based on Exonuclease III-aided Target Recycling Amplification

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Experimental

Chemicals and Materials

The synthetic oligonucleotides DNA purified by PAGE were obtained from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). NMM were purchased from J&K Scientific Ltd. (Beijing, China). Exonuclease III was purchased from Takara Biotechnology Co. Ltd. (Dalian, China). All chemicals were analytical reagent. The water used was purified by Millipore Milli-Q (18 MΩ/cm). Stock solution of oligonucleotides (100 μM) was prepared by deionized water. The stock solution of NMM (6 mM) was prepared in DMSO (dimethyl sulfoxide), stored in darkness at -20°C. Before used, the oligonucleotides solution and NMM were diluted to required concentration with the Tris-HCl buffer (20 mM, pH 7.6).

Fluorometric Analysis

All fluorescence measurements were performed on an F-7000 spectrometer (Hitachi, Japan). The instrument settings were as follows: λ_{EX} = 399 nm (bandpass 10 nm), λ_{EM} from 550 nm to 650 nm (bandpass 10 nm) and the photomultiplier tube (PMT) detector voltage = 500 V. The

target DNA titration was performed by adding 10 fM-100 nM target DNA into 10.0 mM KCl, 10.0 mM $MgCl_2$ and 20 mM Tris-HCl (pH 7.6) buffer containing 500 nM DNA probe, 1 U/μL Exo III and 2.5 μM NMM. All samples were incubated at 37 °C for 120 min. The fluorescence signal change ratios were calculated with the formula $Y=F-F_0$, where F_0 and F are the fluorescence intensities at 615 nm (maximum emission wavelength) in the absence and presence of target DNA, respectively.

Circular Dichroism (CD) Spectra Measurement

5 μM DNA probe in the presence of various molar equivalents of target DNA were measured in 10.0 mM KCl, 10.0 mM $MgCl_2$, 5 U/μL Exo III and 20 mM Tris-HCl (pH 7.6) buffer. The CD spectra were measured using a JASCO J-810 CD spectropolarimeter (Jasco, Japan). The data were recorded for the 220 to 340 nm range at room temperature in a quartz cuvette with a 4 mm optical path length. The data reported herein were averaged from at least 5 scans to improve the signal-to-noise ratio so that the contribution from the buffer was diminished.

Ultraviolet–visible spectroscopy (UV-vis) measurement

The sample was prepared by adding 1 μM DNA probe and 500 nM target DNA into 10.0 mM KCl, 10.0 mM $MgCl_2$, 2 U/μL Exo III and 20 mM Tris-HCl (pH 7.6) buffer. Then the UV-vis spectra were measured using a UV-2600 spectrophotometer (Shimadzu, Japan) at different Exo III reaction time. The data were recorded for the 220 to 340 nm in a quartz cuvette with a 4 mm optical path length. The data reported herein were averaged from at least 3 scans to improve the signal-to-noise ratio so that the contribution from the buffer was diminished.

Table S1. Sequence of oligonucleotides DNA

Name	Sequence
DNA probe	5'-AACCGGGTTTTGGGTTTTGGGTTTTGGGAAAACCCGGTTGAGG-3'
Target DNA (T)	5'-CTTAGCCTCAACCGGGTTTTAGAG-3'
T1	5'-CTTAGCCT**G**AACCGGGTTTTAGAG-3'
T2	5'-CTTAGCCT**GT**ACCGGGTTTTAGAG-3'
T3	5'-CTTAGCCT**GTT**CCGGGTTTTAGAG-3'
T4	5'-CTTAGCCT**GTTG**CGGGTTTTAGAG-3'

Table S2. Compare the capabilities of our method with other methods

Reference	Limit of linear detection /nM	linear detection range /nM
1	0.10	0.25-12.50
2	0.20	0.30-30.00
3	0.50	1.00-20.00
4	7.30	10.00-1000.00
5	3.00	10.00-100.00
Our method	0.76	1.00-100.00

1. N. Xu, J. P. Lei, Q. B. Wang, Q. H. Yang, and H. X. Ju, *Talanta*, **2016**, 661.

2. H. Ma, W. Wei, Q. Lu, Z. Zhou, H. Li, L. Zhang and S. Liu, *Anal. Methods-UK,* **2014**, 6, 6082.

3. S. Wang, Y. Zhang, Y. Ning and G. J. Zhang, *Analyst*, **2015**, 140, 434.

4. L. Liu, Q. Yang, J. Lei, N. Xu and H. Ju, *Chem. Commun. (Camb)*, **2014**, 50, 13698.

5. X. Zhu, H. Zheng, X. Wei, Z. Lin, L. Guo, B. Qiu and G. Chen, *Chem. Commun. (Camb)*, **2013**, 49, 1276.

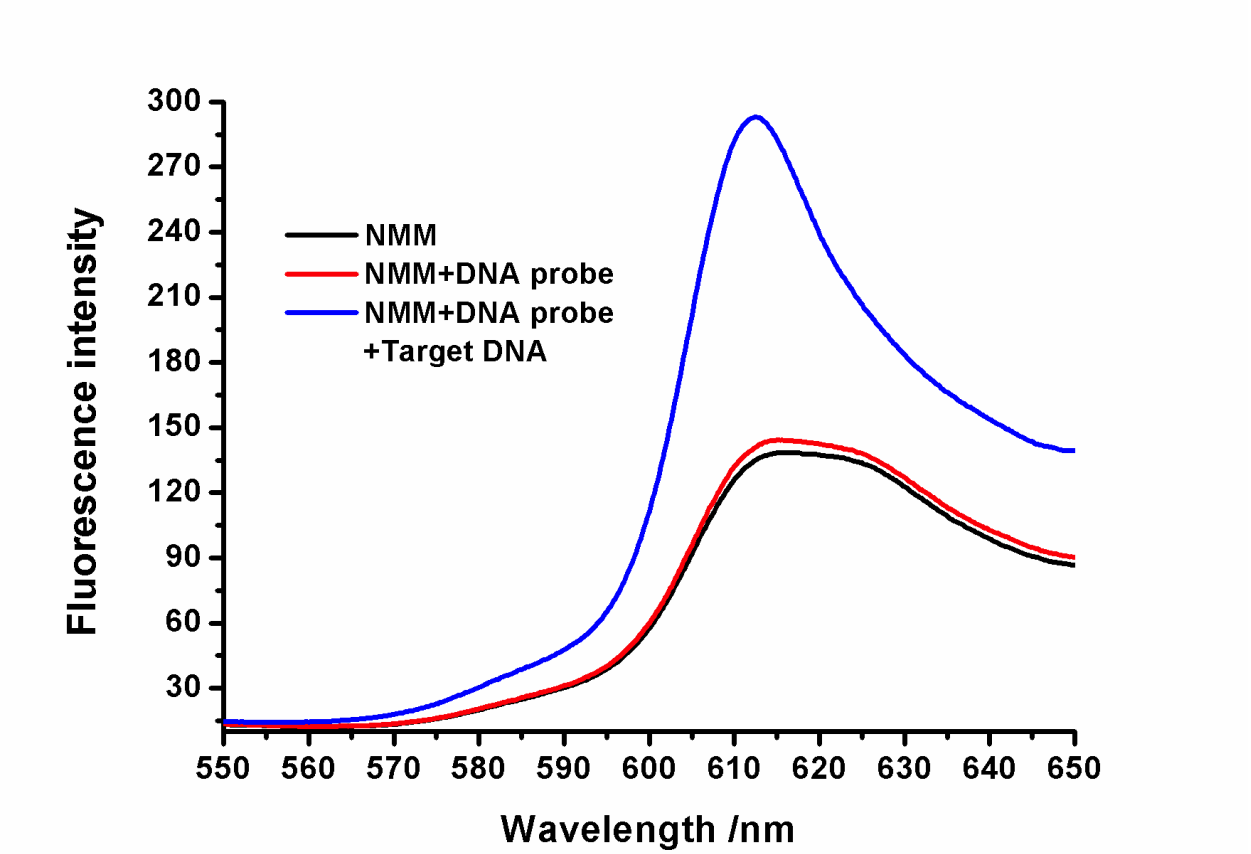

Fig. S1. (a) The fluorescence emission spectrum under different conditions: 2.5 μM NMM (black curve); 2.5 μM NMM + 500 nM DNA probe (red curve); 2.5 μM NMM + 500 nM DNA probe + 100 nM Target DNA (blue curve) in 10.0 mM KCl, 10.0 mM $MgCl_2$, 1 U/μL Exo III and 20 mM Tris–HCl (pH 7.6) buffer.

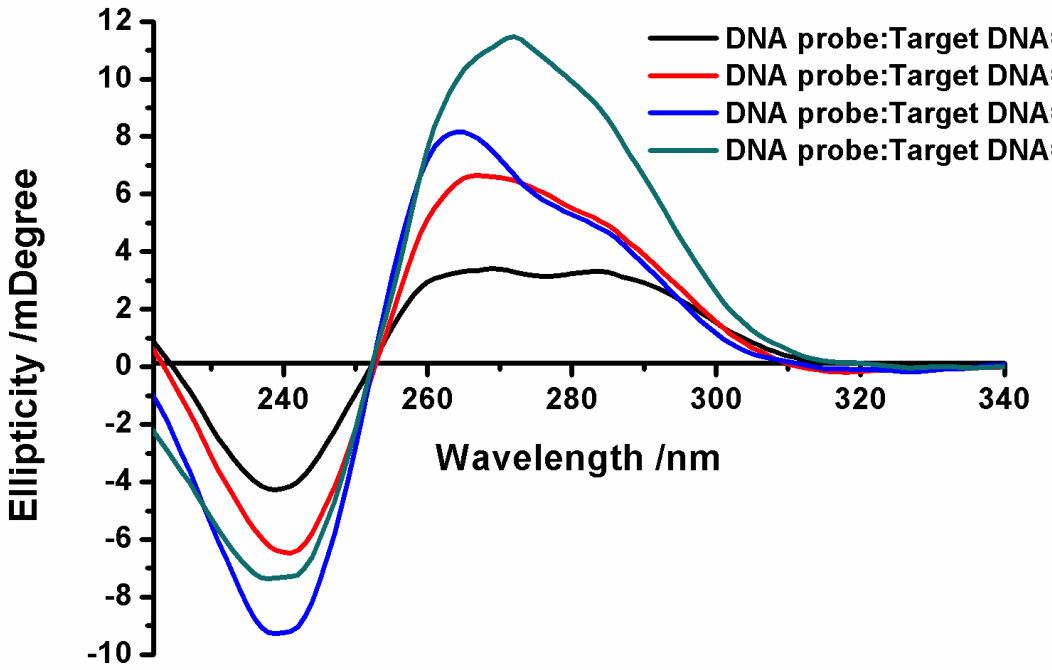

Fig. S2. The CD spectra of 5 μM DNA probe in the presence of various molar equivalents of target DNA in 10.0 mM KCl, 10.0 mM $MgCl_2$, 5 U/μL Exo III and 20 mM Tris–HCl (pH 7.6) buffer. The G-quadruplex structure displayed a characteristic elliptical peak at 272 nm and a trough at 240 nm.

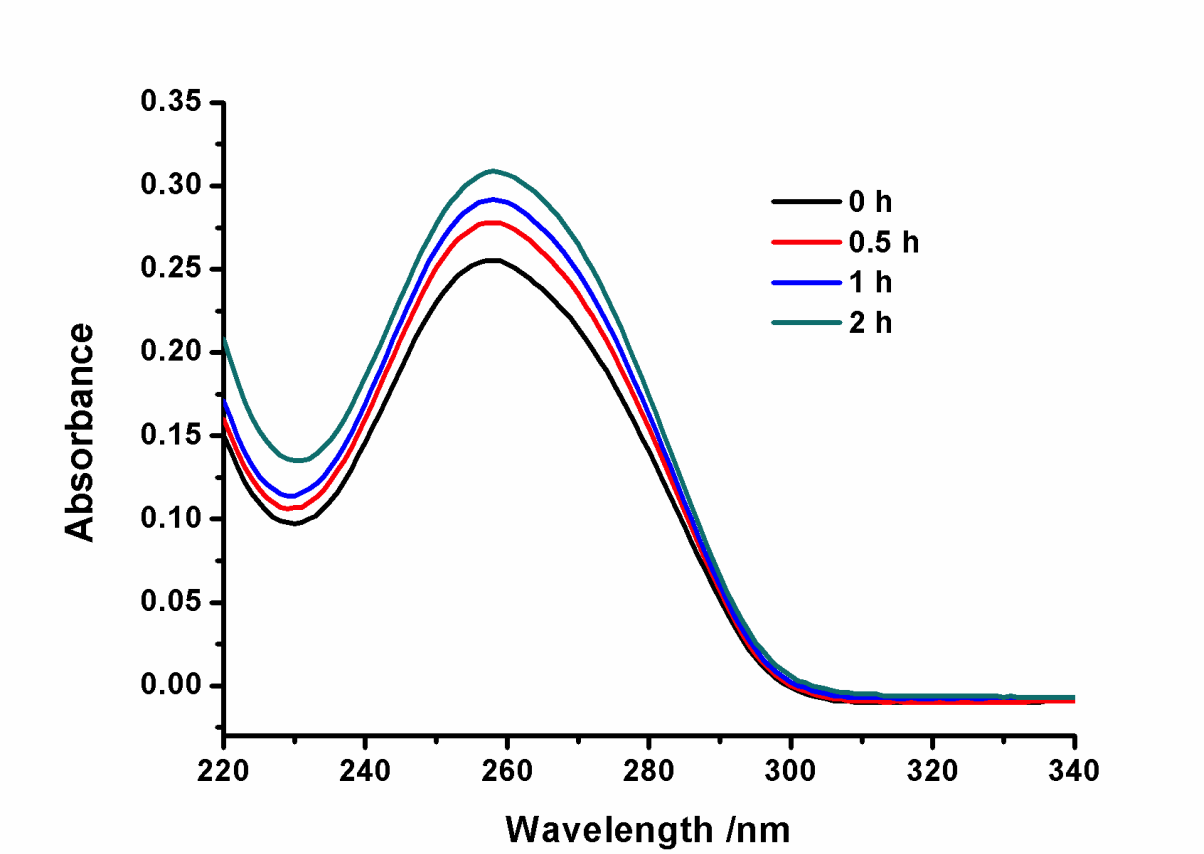

Fig. S3. The UV-vis spectroscopy measurement of 1 μM DNA probe and 500 nM target DNA at different Exo III reaction time in 10.0 mM KCl, 10.0 mM $MgCl_2$, and 20 mM Tris-HCl (pH 7.6) buffer containing 2 U/μL Exo III.

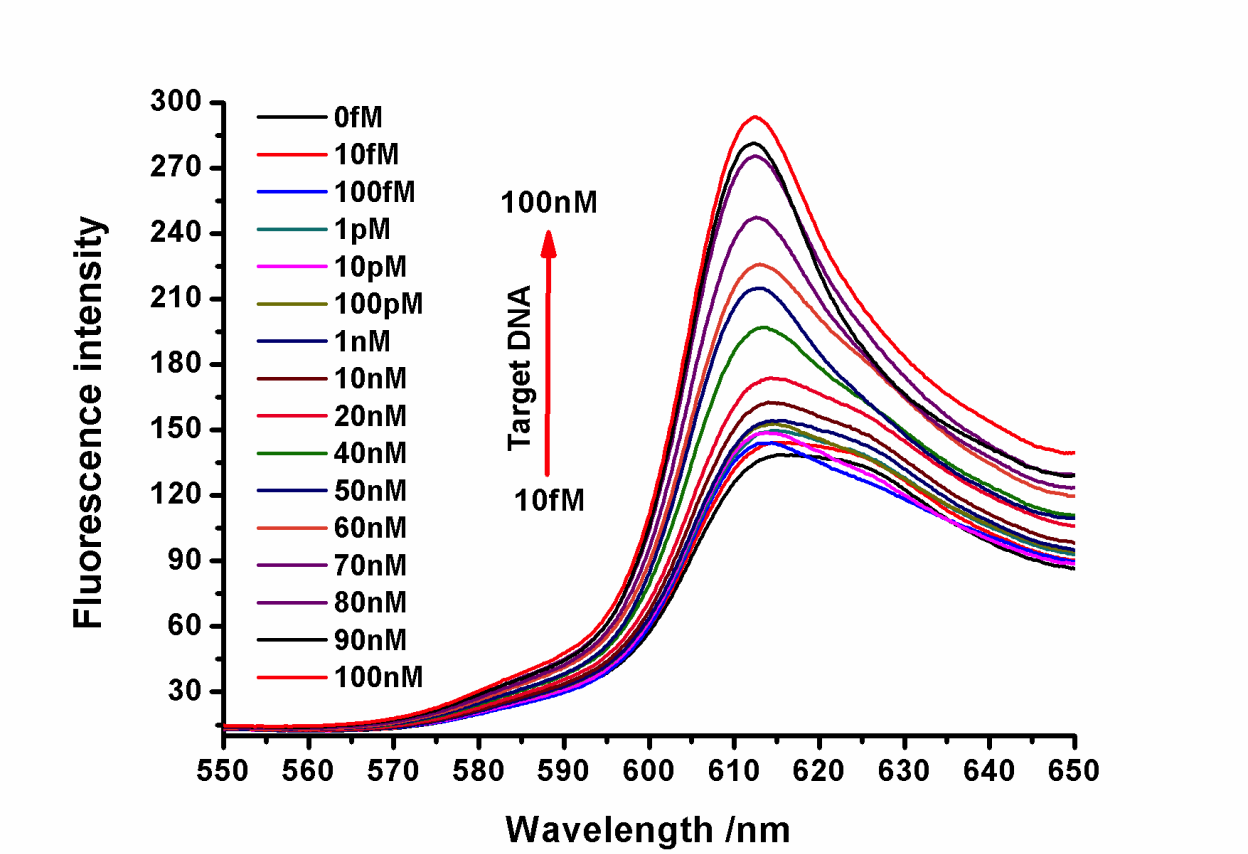

Fig. S4. The fluorescence emission spectra of NMM + DNA probe in the presence of Exo III and various concentrations of target DNA.