Electronic Supplementary Material (ESI) for ChemComm.

Supplementary Information

Real-time monitoring of rolling circle amplification using aggregation-induced emission: applications for biological detection

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1. Experimental details

1.1 Materials and reagents

1,1,2,2-tetrakis{4-[(trimethylammonium)butoxy]pheny} tetraphenylethene tetrabromide (QATPE) was kindly provided by professor Dong-Sheng Guo (Nankai University).[1] SYBR Green I was purchased from Keygen Biotech Co. (Nanjing, China). All of the oligonucleotides (**Pad-lock, LT, PR, ssDNA** and **dsDNA**, Table S1) were purchased from Sangon Biotech. Co. Ltd. (Shanghai, China). The concentrations of the oligonucleotides were represented as single-stranded concentrations. Single-stranded concentrations were determined by measuring the absorbance at 260 nm. Molar extinction coefficient was determined using a nearest neighbor approximation (http://www.idtdan.com/analyzer/Applications/OligoAnalyzer). T4 DNA ligase, Phi29 DNA polymerase, Exonuclease I (Exo I), Exonuclease III (Exo III), restriction endonuclease EcoR I, nicking endonuclease Nb.BbvCl, Taq polymerase, Bst polymerase, deoxyribonucleoside 5′-triphosphate mixture (dNTPs) and bovine serum albumin (BSA) were obtained from New England Biolabs (Beijing, China). All chemical reagents were of analytical grade and used without further purification.

DNAs	Sequence (from 5′ to 3′)
LT	CTCACACGAATTCATCTGAC
Pad-lock	P-5'-ATTCGTGTGAGAAAACGGAACTGCGTCTAGGCAAAAGTCAGATGA-3'
	(P=phosphorylation)
PR	TCATCTGAC
ssDNA	ATTCGTGTGAGAAAACGGAACTGCGTCTAGGCAAAAGTCA
dsDNA	CGACGATGGAATTCTCTTTTGAGAATTCCATCGTCGTTGTT

Table S1 The oligonucleotides used in this work

1.2 Preparation of circular RCA template (cPad-lock).

A mixture of **Pad-lock** (200 nM) and **LT** (600 nM) was prepared in 1 × T4 DNA ligase buffer (50 mM Tris–HCl, 10 mM $MgCl_2$ and 1 mM ATP, pH 7.4). To ensure **Pad-lock** fully hybridized with **LT**, the mixture was heated to 37 ^oC and incubated at this temperature for 0.5 h. Then, 1U T4 DNA ligase was added and the mixture was allowed to incubate at 16 ^oC for 3 h to ensure that the 5′-phosphate and 3′-hydroxyl ends of **Pad-lock** be ligated to form a circular template (**cPad-lock**). The ligation reaction was terminated by a thermal treatment at 65 ^oC for 10 min. Next, 10 U Exonuclease I (Exo I) and 10 U Exonuclease III (Exo III) were added and the mixture was incubated at 37 ^oC for 1 h to digest the leftover single-stranded DNAs and double-stranded DNAs. The prepared circular template (**cPad-lock**) will be used in subsequent experiments.

1.3 Real-time monitoring of RCA reaction.

To above-prepared circular template (**cPad-lock**) was added 40 μM QATPE, different concentrations of **PR**, 0.2 mM dNTPs, 3 μg/mL BSA, 1 × Phi29 buffer (50 mM Tris–HCl, 10 mM $MgCl_2$, 10 mM $(NH_4)_2SO_4$, pH 7.5) and 2U Phi29 DNA polymerase. The total volume was 35 μL. The RCA reaction was performed at 30 ^oC in a commercial StepOnePlus^{TM} Real-Time PCR instrument (ABI company, USA) for 5 h. The fluorescence detection channel set for FAM (6-carboxylfluorecein) was used for fluorescence signal collection. The fluorescence signal was collected at intervals of 2 min.

1.4 End-point detection of RCA product

When the detection of RCA product was conducted by the end-point mode, 40 μM QATPE was added after RCA reaction instead of before RCA reaction. The final volume was 100 μL. The fluorescence signal was recorded in a SHIMADZU RF-5301PC spectrofluorimeter with 1cm-path-length micro quartz cell (40 μL, Starna

Brand, England). The excitation and emission wavelengths were set at 330 and 475 nm, respectively.

1.5 Gel electrophoresis

The RCA reaction mixtures (25 μL total volume) were sufficiently mixed with 200 μM QATPE. Then, 5 μL loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol, 10 mM EDTA) was added. The mixtures were loaded onto a 1% agarose gel, and electrophoresis analysis was carried out in TBE buffer (89 mM Tris-boric acid, 2.0 mM EDTA, pH 8.3) at room temperature. The gel was run at a constant potential of 70 V for 1 h, and was photographed by a Gel Documentation system (Huifuxingye, Beijing, China).

1.6 AFM imaging

Freshly cleaved mica was soaked in 10 mM $MgCl_2$ for five minute, then dried with N_2. The samples were diluted 100-fold in deionized H_2O and 5 μL was applied to the mica for 30 seconds, rinsed with dH_2O and dried with N_2 again. The samples were imaged in tapping mode with a Digital Instruments scanning probe microscope (Dimension 3100) with Nanoscope IIIa controller hardware.

1.7 T4 DNA ligase-sensing based on real-time RCA.

Circular RCA template was prepared as above but different concentrations of T4 DNA ligase were added. Then, 40 μM QATPE, 10 nM **PR**, 0.2 mM dNTPs, 3 μg/mL BSA, 1 × Phi29 buffer and 2U Phi29 DNA polymerase were added. RCA reaction was conducted performed at 30 ^{o}C in the StepOnePlus^{TM} Real-Time PCR instrument for 5 h, and fluorescence signal change of QATPE was recorded as a function of reaction time. T4 DNA ligase activity was determined by three ways using fluorescence signal at designated time, initial reaction rate, or the data processing method provided by the commercial real-time PCR instrument, respectively.

2. Different discriminating abilities of QATPE and SG I towards ssDNA and dsDNA

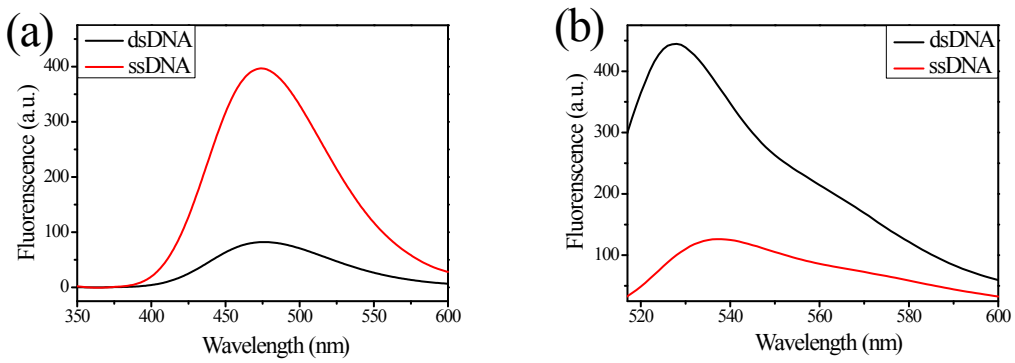


Figure. S1 Fluorescence emission spectra of (a) QATPE and (b) SG I in the presence of ssDNA or dsDNA.

3. Fluorescence response rate of QATPE to single-stranded DNA product of RCA

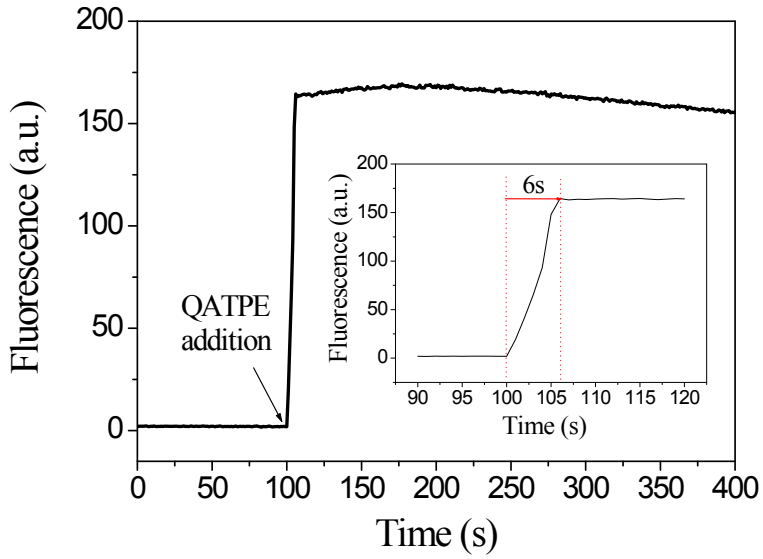


Figure. S2 Fluorescence signal change with time after mixing of QATPE with RCA product. [PR] =10 nM. [QATPE] = 40 nM.

4. Real-time monitoring of RCA reactions containing deferent concentrations of primer.

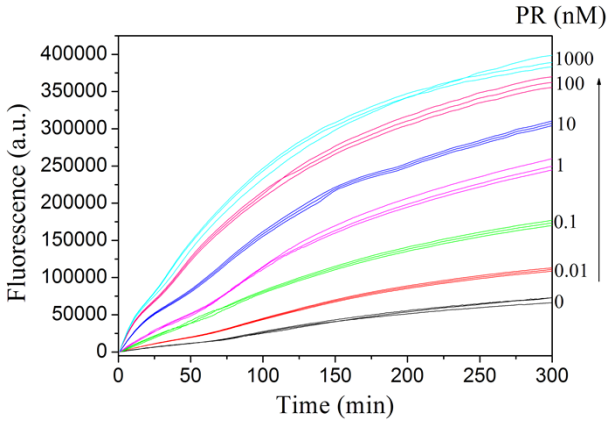
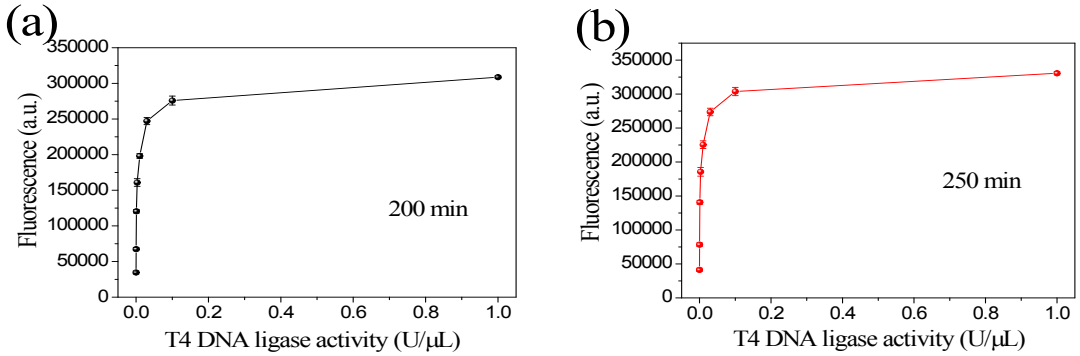


Figure S3 Time-dependent fluorescence changes of the RCA reaction solutions containing different concentrations of **PR**. All experiments were performed in triplicate.

5. End-point detection of T4 DNA ligase activity using the experimental results at different time points.



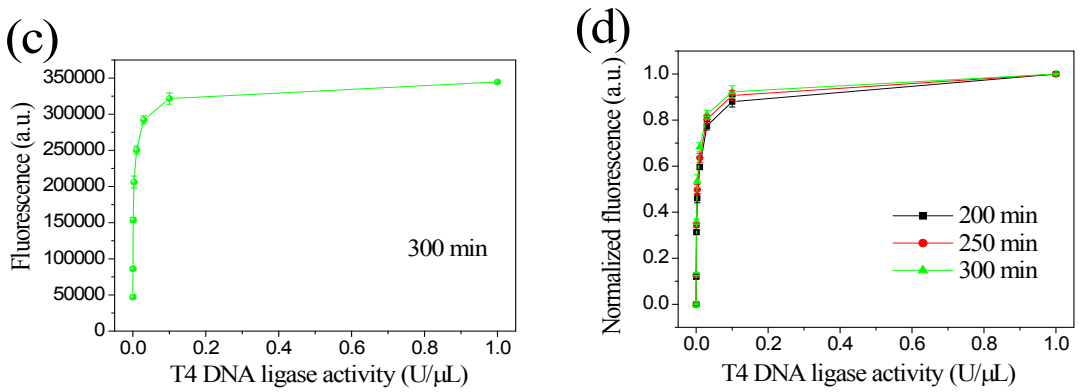


Figure S4 fluorescence signal change at 200 min (a), 250 min (b) or 250 min (c) as a function of T4 DNA ligase activity; (d) Normalized fluorescence signal changes at the three time points as a function of T4 DNA ligase activity.

6. Selectivity of T4 DNA ligase sensor

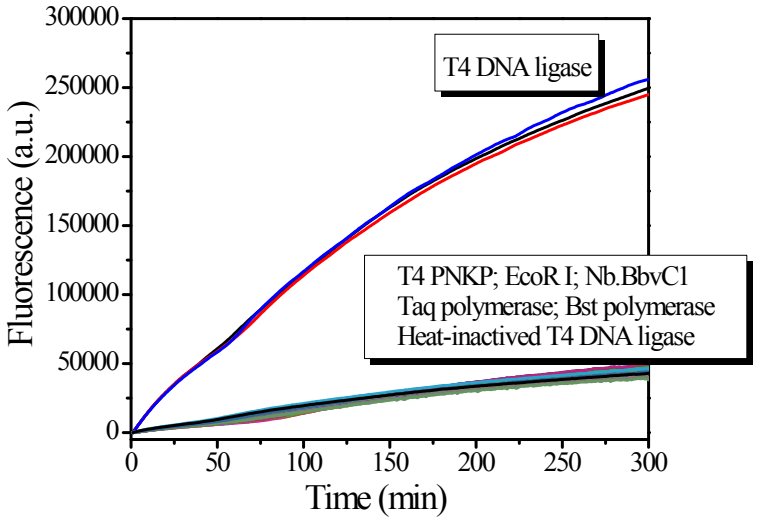


Figure. S5 Fluorescence responses of the proposed T4 DNA ligase sensor to T4 DNA ligase and other enzymes. [T4 DNA ligase] = 0.01 U/μL; [T4 PNKP] = [EcoR I] = [Nb.BbvCl] = [Taq polymerase] = [Bst polymerase] = 0.1 U/μL. Heat-inactived T4 DNA ligase was prepared by heating the enzyme at 95 °C for 10 min. All experiments were performed in triplicate.

References

1 B. P. Jiang, D.S. Guo, Y. C. Liu, K. P. Wang, Y. Liu, ACS Nano. 2014, 8, 1609-1618.