Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Experimental details

Materials and Measurements

The nicking enzyme (N.BstNB I) and 10×NEBuffer 3 (50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM dithiothreitol, pH 7.9) were obtained from New England BioLabs.

All DNA sequences were synthesized and purified by Sangon Biotech Co., Ltd. (Shanghai, China), and their base sequences in detail were as follows：

Hairpin capture probe:

5′-*CCCTACCC***GAGTC**TTCC↓AGTGTGATGA*GGGTAGGG*CGGGTTGGG-3′,

19-nt sense sequence of p53 gene target: 5′-TCATCACACTGGAAGACTC-3′

single-base mismatched sequence: 5′-TCATCACACTGGAAGAATC-3′.

In the probe, the italic letters at the two ends represent the sequence of the stem arms; the bold letters are the recognition sequence of N.BstNB I, and the arrow indicates the nicking position; the underlined letters are a classical sequence of G-quadruplex. In the target, the underlined letter is the 259 codon of p53, and the mutation of C→A could induce the change of amino acid from Aspartic acid to Glutamic acid.

ABTS (2, 2-azinobis(3-ethylbenzothiozoline)-6-sulfonic acid), Hemin, H_2O_2, and HEPES (4-(2-hy-droxyethyl) piperazine-1-ethanesulfonic acid sodium salt) were purchased from Aladdin Reagents (Shanghai, China) and used as supplied. Hemin

stock solution (1 mM) was prepared in dimethylsulfoxide (DMSO) and stored at -20℃. Hemin, $ABTS^{2-}$ and H_2O_2 were freshly prepared before used.

Ultrapure water was obtained from a Milli-Q Millipore filtration system and used throughout. UV-vis absorption spectra were obtained on a Lamda 750 UV-Vis spectrophotometer (PE, USA).

Nicking endonuclease amplification reaction

In a typical procedure, a solution containing 5μL 10×NEBuffer 3, 10μL of 1 μM hairpin capture probe and a specific concentration of target sequence (0 pM, 1 pM, 5 pM, 10 pM, 50 pM, 100 pM, respectively) was added to a microtest tube. After a certain time of hybridization, a nicking enzyme (N.BstNB I, 10 units/μL) was added to yield a total volume of 100 μL, and incubated at 55℃ for 60 min. Finally, the resulting mixtures were heated at 90℃ for 10 min to deactivate the nicking enzyme. For control experiment, the single-base mismatched sequence was used.

Colorimetric Measurement

5 μL of 1 μM Hemin was added to the mixtures, followed by the addition of buffer (25 mM HEPES, 20 mM KCl, 200 mM NaCl, 0.05% Triton X-100 and 1% DMSO; pH 8.0) to a total volume of 200 μL. The mixtures were incubated for 60 min, and then added by ABTS and H_2O_2 such that a final concentration of the solution reaches 2 mM. Finally, the UV absorption spectra were recorded by a Lambda 750 UV-Vis spectrophotometer (λ=419 nm).

Control experiments

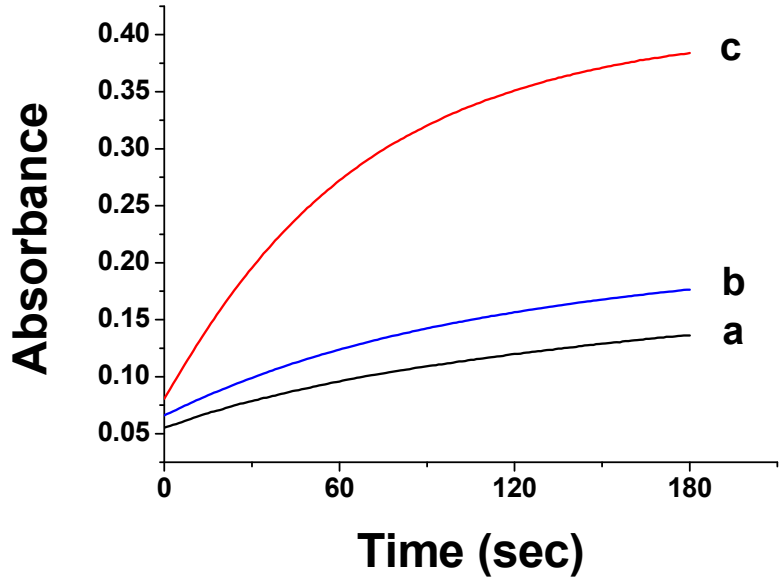

Figure S1. Time- dependent absorption intensity at 419 nm from the oxidation of ABTS. (a) Without target DNA, (b) 100 pM target p53 gene incubated without endonuclease and (c) 100 pM target p53 gene incubated with endnuclease for signal amplification. Colorimetric measurements were performed in the solution containing 25 nM hemin, 2 mM $ABTS^{2-}$ and 2 mM H_2O_2.

To investigate the feasibility of the method described in this work, a variety of control experiments were conducted. As shown in Fig. S1, the absorbance obtained after the cycles of cleavage(curve c) is nearly 3-fold higher than that of without the process of signal amplification(curve b). This confirms endonuclease plays a key role in signal amplification. Compared with the background(curve a), the signal gotten from a perfectly matched target (curve b) only increased little if the solution contained no endonuclease. In addition, the process of released G-rich sequences combined with hemin to form catalytically active DNAzyme had been taken into account. If no K^+ or Na^+ presented in the solution, the G-quadruplex was difficult to form, and nearly no color change was observed, which was consistent with the previous reports[1, 2]. This

indicated that the formation of G-quadruplex was the sticking point of the proposed method.

References

1. P. Travascio, Y. Li and D. Sen, *Chem. Biol.*, 1998, **5**, 505-517.
2. D. M. Kong, L. L. Cai, J. H. Guo, J. Wu and H. X. Shen, *Biopolymers*, 2009, **91**, 331-339.