Electronic Supplementary Information

S1. Experimental section

Aptasensor for thrombin. Firstly, each well of the microtiter plates was incubated with 200 μL of glutaraldehyde (5 mM) at 37 °C for 4 h. After washing the plates three times with PBS buffer (pH 5.0) and twice with deionized water, the plates were modified with primer DNA (1) by incubating 200 μL of DNA (1) (1 μM) in TE buffer (pH 7.4) at 37 °C for another 4 h (The sequences of DNA (1) and (2) are listed in Table S1). Subsequently, the plates were washed three times with deionized water, followed by adding 25 μL of 1 μM circle template (2) and 3 μL of 10×T4 ligase buffer. After heating at 90 °C for 10 min and cooling down to room temperature for 1 h, 2 μL of T4 ligase (20 U) was added and reacted at 25 °C for 2 h to make DNA (2) circular. And then, the plates were washed three times with PBS buffer, and 40 μL of deionized water, 5 μL of 10×phi29 reaction buffer and 1 μL of thrombin with different concentrations (0 M,10^{-17} M, 5 × 10^{-17} M, 10^{-16} M, 5 × 10^{-16} M, 10^{-15} M) were added to each well. After 15 min, 3 μL of dNTPs (10 mM) and 1 μL of phi29 DNA polymerase (10 U) were added to the mixture to perform the RCA reaction at 37 °C for 3 h. Finally, the liquid were removed and the plates were washed three times, followed by incubating with 100 μL of hemin (5 μM) at 25 °C for 1h.

Table S1. DNA sequences used in this study

Name	Sequence (5′-3′)
1	P-CTTCGCCGTCCCCAACCCGCCCTACCCGGTTGGTGTGGTTGGCCCAACCCGCCCTACCCAGTATCAATC
2	NH_2-GACGGCGAAGGATTGATACT
HRP-mimicking DNAzyme	GGGTAGGGCGGGTTGGG

Growth of gold nanoparticles for signal generation. After removing the liquid and washing the plates three times with deionized water, 100 μL of hydrogen peroxide (6 mM) in 1 mM MES buffer (pH 6.5) was added to each well and reacted for 30 min at room temperature. Then, 100 μL of freshly prepared gold (III) chloride trihydrate (0.2 mM) in MES buffer was added. The photographs and UV-vis absorbance were recorded after reaction for 15 min.

Native polyacrylamide gel electrophoresis (PAGE). The 15% non-denaturing PAGE was prepared by mixing 4 mL of 30% acrylamide/bis-acrylamide gel solution (29:1), 160 μL of 50×TAE/Mg^{2+} buffer, 80 μL of 10% ammonium persulfate (APS), 4 μL of N,N,N',N'-tetramethylethylenediamine (TEMED) and 3756 μL of deionized water. A 15 μL of each sample was mixed with 2 μL of 10× loading buffer, followed by pipetting the mixture into the 15% gel for electrophoresis. The PAGE was running at room temperature at 170 V for 6 min and 110 V for 40 min. And the the gel were stained in diluted 4S Red Plus (Shanghai Sangon Biotech, China) for 30 min and scanned on an automatic digital gel imaging analysis system (Tanon 2500R, Shanghai, China).

S2. Calculation of coverage of DNA (1) on each well

DNA (1) was immobilized on 96-well plates according to the procedures described in experimental section. To estimate the coverage of DNA (1) on each well, firstly, we obtained the UV-vis calibration curve of DNA (1) in the range from 1.0×10^{-8} to 1.0×10^{-6} M (Figure S1). The coverage of DNA (1) on each well was quantitatively evaluated from the differences of UV-vis absorbance of DNA (1) solution at 258 nm before and after its immobilization as follows.

The concentration of DNA (1) before immobilization, $C_0 = 1 \times 10^{-7}$ M

The concentration of DNA (1) after immobilization in suspension, $C_1 = 9.516 \times 10^{-8}$ M

The volume of DNA (1) solution pipetted into each well, V = 2 mL

Thus, the moles of total DNA (1) immobilized on each well, $n = (C_0 - C_1) \times V = (1 \times 10^{-7} - 9.516 \times 10^{-8}) \times 2 \times 10^{-3} = 9.68 \times 10^{-12}$ mol

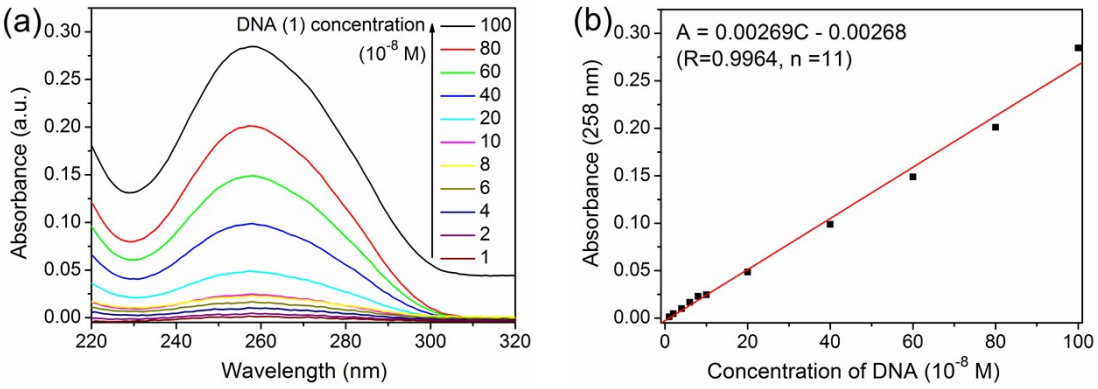
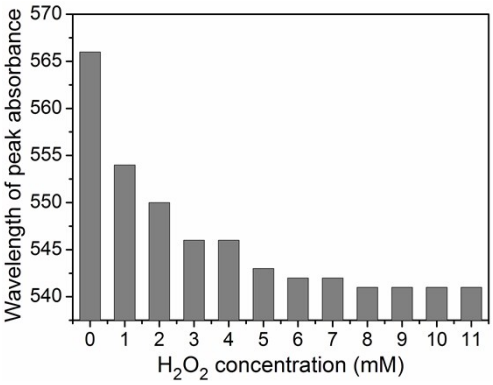


Fig. S1 (a) UV-vis spectra and (b) corresponding calibration curve of standard DNA (1) solution.

S3. The wavelength of peak absorbance corresponding to different concentrations of hydrogen peroxide in Fig. 2 of the main text.



S4. The influences of hemin/G-quadruplex HRP-mimicking DNAzyme and hemin on AuNP growth

According to equation (2) in Scheme 1b, HRP-mimicking DNAzyme can catalyze the decomposition of hydrogen peroxide into H_2O and O_2. The influences of hemin/G-quadruplex HRP-mimicking DNAzyme and hemin on AuNP growth were also investigated (see ESI). In comparison with hemin, the biocatalytic activity is significantly enhanced upon intercalating hemin with a guanine-rich single-stranded nucleic acid to generate a HRP-mimicking DNAzyme with G-quadruplex structure. From Fig. S2, as the concentration of hydrogen peroxide is constant, the color of the nanoparticle dispersion changes from red to blue with increasing the concentration of HRP-mimicking DNAzyme from 0 to 5 nM, resulting from the high catalytic activity of DNAzyme that reduces the concentration of hydrogen peroxide below 3 mM. In contrast, the solution remains a red color for hemin at the same concentration range due to the low catalytic action of hemin that makes hydrogen peroxide in the growth solution at high levels.

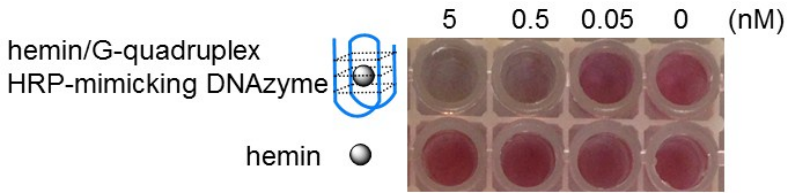


Fig. S2 Photograph of the influence of hemin/G-quadruplex HRP-mimicking DNAzyme and hemin on the generation of gold nanoparticles. The final concentrations of hydrogen peroxide and Au^{3+} are 3 mM and 0.1 mM, respectively, in 1 mM MES buffer (pH 6.5).

S5. Comparison of this work with other AuNP-based colorimetric methods for thrombin detection

Strategy	Detection limit	Ref.
Aptamer-AuNPs conjugates based on a dot-blot array	14 fmol	1
Aptamer-based colorimetric sensing using unmodified AuNPs	0.83 nM	2
Turn-on colorimetric sensor using fibrinogen–gold nanoparticle conjugate	0.16 amol	3
Molecularly imprinted aptamers of AuNPs	0.5 nM	4
One-pot synthesized DNA-templated Ag/Pt bimetallic nanoclusters as peroxidase mimics	2.6 nM	5
A plasmonic aptasensor via arrested rolling circle amplification	10 aM	this assay

S6. Comparison of this work with other RCA strategies for thrombin detection

Strategy	Detection limit	Ref.
Triggered polycatenated DNA scaffolds via RCA and DNAzyme amplification by chemiluminescence detection	6.6 pM	6
Arrest of RCA by protein-binding DNA aptamers by colorimetric method	15 nM	7
Surface plasmon resonance sensors by aptamer-based RCA and nanoparticle signal enhancement	0.78 aM	8
Fluorescence aptameric sensor based on hyperbranched RCA	2 fM	9
Hyperbranched RCA based electrochemiluminescence aptasensor	1.2 aM	10
A Portable colorimetric microchip by RCA and hemin/G-quadruplex system	83 fg/mL	11
Surface enhanced Raman spectroscopy detection based on DNAzyme assistant DNA recycling and RCA	2.3 fM	12
A plasmonic aptasensor via arrested rolling circle amplification	10 aM	this assay

References

1 Y. Wang, D. Li, W. Ren, Z. Liu, S. Dong and E. Wang, *Chem. Commun.*, 2008, 2520-2522.

2. H. Wei, B. Li, J. Li, E. Wang and S. Dong, *Chem. Commun.*, 2007, 3735-3737.

3. Y. Niu, P. Wang, Y. Zhao and A. Fan, *Analyst*, 2013, **138**, 1475-1482.

4. Y. -J. Liao, Y.-C. Shiang, C.-C. Huang and H.-T. Chang, *Langmuir*, 2012, **28**, 8944-8951.

5. C. Zhang, A.-X. Zheng, B. Liu, X.-L. Zhang, Y. He, J. Li, H.-H. Yang and G. Chen, *Chem. Commun.*, 2014, **50**, 13103-13106.

6. S. Bi, L. Li and S. Zhang, *Anal. Chem.*, 2010, **82**, 9447-9454.

7. L. Wang, K. Tram. M. M. Ali, B. J. Salena, J. Li and Y. Li, *Chem. Eur. J.*, 2014, **20**, 2420-2424.

8. P. He, L. Liu, W. Qiao and S. Zhang, *Chem. Commun.*, 2014, **50**, 1481-1484.

9. X. Zhu, H. Xu, H. Zheng, G. Yang, Z. Lin, B. Qiu, L. Guo, Y. Chi and G. Chen, *Chem. Commun.*, 2013, **49**, 10115-10117.

10. G. Jin, C. Wang, L. Yang, X. Li, L. Guo, B. Qiu, Z. Lin and G. Chen, *Biosens. Bioelectron.*, 2015, **63**, 166-171.

11. X. Lin, Q. Chen, W. Liu, H. Li and J.-M. Lin, *Biosens. Bioelectron.*, 2014, **56**, 71-76.

12. F. Gao, L. Du, D. Tang, Y. Lu, Y. Zhang and L. Zhang, *Biosens. Bioelectron.*, 2015, **66**, 423-430.