Supporting Information

DNA flower-encapsulated horseradish peroxidase with enhanced biocatalytic activity synthesized by an isothermal one-pot method based on rolling circle amplification

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Additional experimental section

Chemicals and materials. T4 DNA ligase, exonuclease I (Exo I), phi29 DNA polymerase, 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulfonate diammonium salt (ABTS), 2-(N-morpholino)ethanesulfonic acid (MES) and 4S Red Plus solution were purchased from BBI Life Sciences Co., Ltd. (Shanghai, China). Bradford protein assay kit and deoxynucleotides (dNTPs) were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, China). Horseradish peroxidase (HRP), hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), silver nitrate ($AgNO_3$), chloroauric acid tetrahydrate ($HAuCl_4 \cdot 4H_2O$), cetyltrimethylammonium bromide (CTAB), sodium borohydride ($NaBH_4$) and L-ascorbic acid (AA) were provided by Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 1-ethyl-(3-3'-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) were ordered from Aladdin Reagent Co., Ltd. (Shanghai, China). Thrombin was provided by Macklin Biochemical Co., Ltd. (Shanghai, China). The oligonucleotides used in this work were synthesized and HPLC-purified by Sangon Biotechnology Co., Ltd. (Shanghai, China), and the sequences are listed in Table S1. PicoGreen dsDNA reagent was purchased from Yeasen Biotech Co., Ltd. (Shanghai, China). Carboxyl-modified magnetic microparticles (MMPs) (3~4 μm，0.5% W/V) were obtained from BaseLine ChromTech Research Centre (Tianjin, China). All reagents were of analytical grade and used without further purification. Nuclease-free ultrapure water was used in the whole experiments.

Table S1. Sequences of oligonucleotides used in this work

Name	Sequences (5'-3')[a]
padlock probe	phosphate- AATATTATTCCAGCTGGCAGTCACCCCAACCTGCC CTACCACGGACTGACTGCACCTTGAACGCTTATTATGATT
primer	CTGGAATAATATT AATCATAATAAGC
Apt15	GGT TGG TGT GGT TGG-NH_2

[a] The underlined sequence in padlock probe is complementary to the sequence of Apt29.

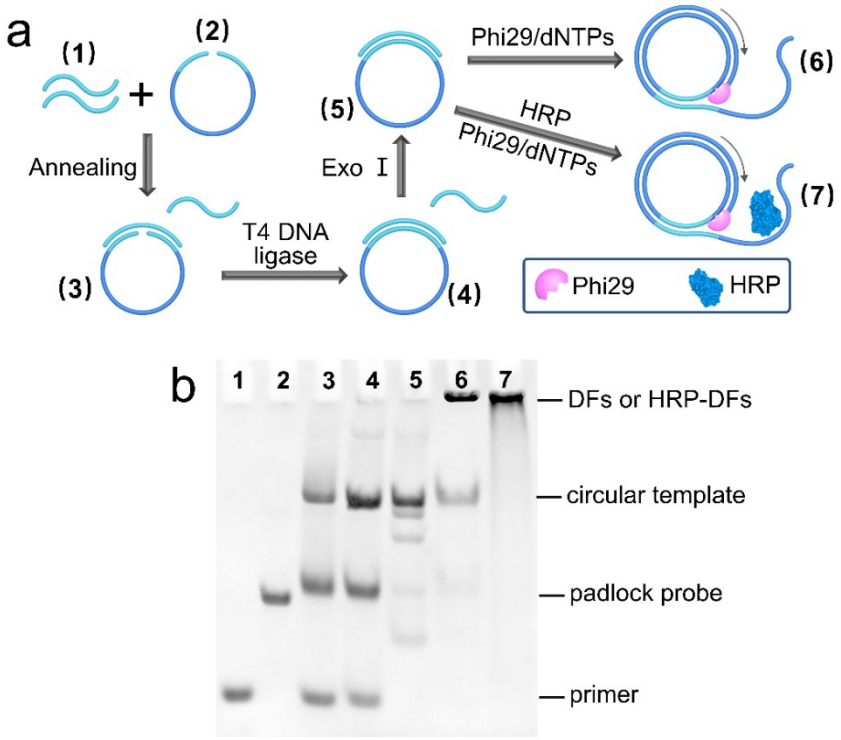

Fig. S1 Non-denaturing PAGE characterization. (a) Schematic illustration of circular template preparation for RCA reaction in the absence and presence of HRP. (b) Non-denaturing PAGE image. Lane 1: primer, lane 2: padlock probe, lane 3: padlock probe/primer hybrid after annealing, lane 4: sample in lane 3 treated with T4 DNA ligase, lane 5: sample in lane 4 treated with Exo I, lane 6: DFs, and lane 7: HRP-DFs.

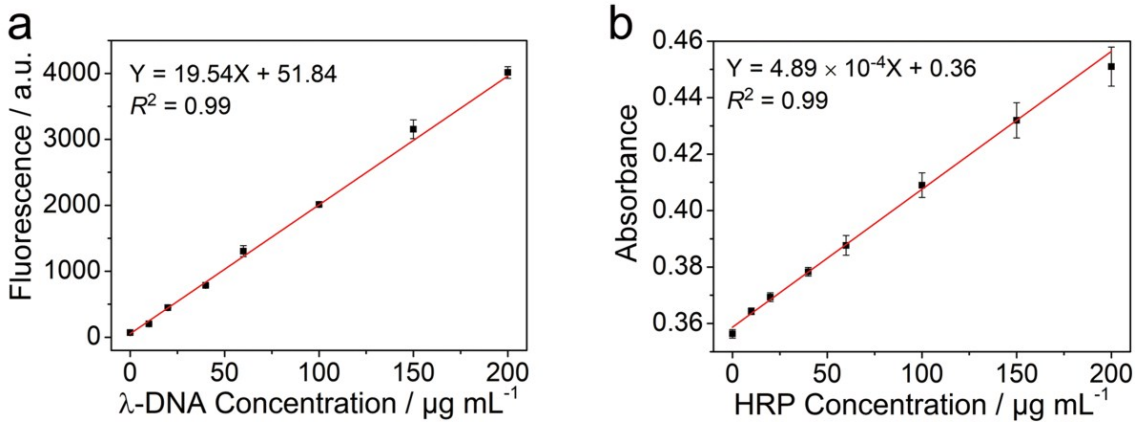

Fig. S2 Standard curves of (a) DNA measured by λ-DNA and (b) protein measured by HRP. The curves are obtained from three independent measurements.

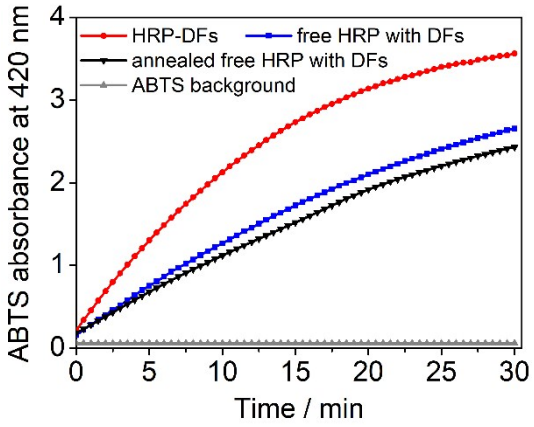

Fig. S3 Dynamic UV-vis absorbance of ABTS at 420 nm catalyzed by HRP-DFs, free HRP with DFs, and annealed free HRP with DFs.

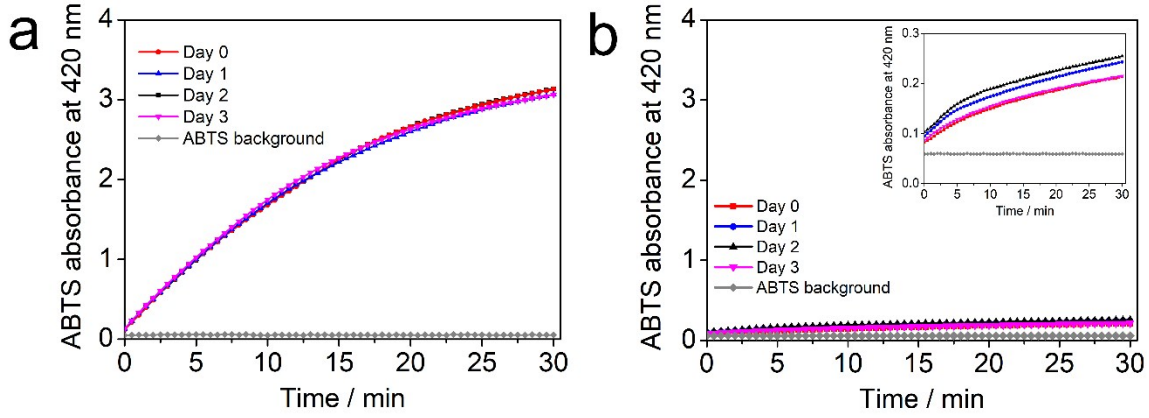

Fig. S4 Dynamic UV-vis absorbance of ABTS at 420 nm catalyzed by (a) HRP-DFs and (b) supernatant of HRP-DFs on different days.

Table S2 Recovery of thrombin detected in diluted human serum samples by the proposed aptasensor

sample	added (fM)	founded (fM)	recovery (%)	RSD (%)[b]
1	0	–[a]	–[a]	–[a]
2	10	9.83	98.3	6.0
3	50	49.42	98.8	2.9
4	70	69.74	99.6	3.9

[a] "–" denotes that the concentration of the analyte is too low to be detected.

[b] The results are obtained from five independent experiments.

Table S3 Comparison of the proposed assay with other methods for thrombin detection

Strategy	Detection technique	Linear range	LOD	Ref.
Magnetic force assisted aptamer-antibody sandwich assay	Electrochemistry	1 nM-500 nM	0.49 nM	[1]
HCR and DNA triplex assembly	Fluorescence	0.5 nM-200 nM	0.32 nM	[2]
Molecular machine powered surface programmatic chain reaction	Electrochemistry	2 pM-20 nM	0.76 pM	[3]
$_{L}$-Cys-hemin/G-quadruplex-based self-catalytic platform	Electrochemistry	0.1 pM-80 nM	0.032 pM	[4]
HCR-mediated assembly of bioluminescent modules and self-illumination-based BRET	Bioluminescence	14.4 pM-9 nM	12.8 pM	[5]
TSDR-mediated proximity binding and recycling amplification	Electrochemistry	0.05 nM-100 nM	23.6 pM	[6]
Dual-signaling electrochemical ratiometric amplification and DNA walker recycling amplification	Electrochemistry	0.1 pM-10 pM	56 fM	[7]
DNA enzyme/AuNPs-based dual-signal amplification	Photoelectrochemistry	87.5 fM-8.75 nM	17.3 fM	[8]
DNA flower-encapsulated HRP with enhanced biocatalytic activity for etching of AuNRs	Visual and colorimetric detection	5 fM-80 fM	0.22 fM	This work

HCR, hybridization chain reaction; BRET, bioluminescence resonance energy transfer; TSDR, toehold-mediated strand displacement reaction

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