Supporting Information

Reporter-triggered Isothermal Exponential Amplification Strategy in Ultrasensitive Homogeneous Label-free Electrochemical Nucleic Acid Biosensing

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Experimental Section

Reagents and Apparatus

All HPLC-purified DNA oligonucleotides (listed in Table S1) were synthesized by Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China). The vent (exo-) DNA polymerase and nicking endonuclease Nt.BstNBI were purchased from New England Biolabs (Beverly, MA). The deoxynucleotide mixture (dNTPs) was bought from Takara Biotechnology Co. Ltd (Dalian, China). Hydrogen peroxide (H_2O_2) and Triton X-100 were purchased from Beijing Chemical Reagent Company (Beijing, China). Hydroquinone (HQ) was purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). HRP (Peroxidase Horseradish, E.C.1.11.1.7, ≥250 units/mg, RZ 3.0), hemin, potassium chloride (KCl) and magnesium chloride ($MgCl_2$) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hemin was prepared in dimethyl sulfoxide and stored at -20 ℃ as 5 mM stock solution. All other reagents were at least of analytical reagent grade. Human serum samples were kindly provided by Peking University Hospital (Beijing, China). Ultrapure water from a Milli-Q water purification system (Bedford, MA, U.S.A) was used throughout.

The isothermal amplification reaction was carried out by TC-512 PCR (TECHNE, UK). The electrochemical experiments were performed by a CHI 660C electrochemical workstation (Shanghai Chenhua Instruments Company, Shanghai. China). The measurements were based on a homemade ultramicroelectrode/micropipette-tip miniaturized electrochemical device.

Reporter-triggered Isothermal Exponential Amplification Reaction (R-EXPAR)

The R-EXPAR was prepared separately as part A and part B solutions. Part A consisted of Y′-Y′ template, Reporter EAD2 sequence, Nt.BstNBI buffer and dNTPs. Part B consisted of the nicking endonuclease Nt.BstNBI, vent (exo-) DNA polymerase and ThermoPol buffer. Part A and Part B were mixed immediately containing Y′-Y′ template (100 nM), different concentrations of EAD2, Nt.BstNBI (0.4 U/μL), vent (exo-) DNA polymerase (0.05 U/μL), dNTPs (375 μM), 1 ×ThermoPol buffer (20 mM Tris-HCl, pH 8.8, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$, 0.1% Triton X-100) and 0.5 × Nt.BstNBI buffer (25 mM Tris-HCl, pH 7.9, 50 mM NaCl, 5 mM

$MgCl_2$, 0.5 mM DTT). The mixture at a volume of 10 μL was incubated at 55 ℃ for 15 min and quenched at 4 ℃ for further measurement.

X′-Y′/ Y′-Y′ strategy for DNA Assay

X′-Y′ strategy was performed at a volume of 10 μL containing X′-Y′ template (200 nM), dNTPs (375 μM), Nt.BstNBI (0.4 U/μL), vent (exo-) DNA polymerase (0.05 U/μL), different concentrations of target X, 1 ×ThermoPol buffer and 0.5 × Nt.BstNBI buffer. The mixture was incubated at 55 ℃ for 60 min and stored at 4 ℃. Then, 1 μL reaction sample was added into R-EXPAR system (following the above protocol) and incubated at 55 ℃ for 8 min. The X′-Y′/Y′-Y′ product was kept at 4 ℃ for subsequent electrochemical analysis.

Electrochemical Measurements

A homemade ultramicroelectrode/micropipette-tip miniaturized electrochemical device had been constructed in our previous work.[14] A carbon fiber ultramicroelectrode with the surface area of 6.25×10^{-10} m^2 (working electrode, WE) was inserted into a micropipette tip containing analyte solution with fixed volume. The WE, Ag/AgCl reference electrode (RE) and Pt counter electrode (CE) were assembled in a homemade cell containing phosphate buffer to establish a three-electrode system.

A 5 μL reaction sample was mixed with 5 μL 50 μM hemin, 5 μL 4 mM HQ and 5 μL 4 mM H_2O_2 (All prepared in phosphate buffer, 0.1 M NaH_2PO_4-Na_2HPO_4, pH 7.4, 5 mM KCl, 5 mM $MgCl_2$). A 20 μL solution indrawing in micropipette tip was used for performing electrochemical measurement via the above electrochemical device.

Table S1 DNA sequences used in this work

Name	Sequence(5'- to 3'-)
Y′-Y′	TCCCTCCCTCCCTCCCAGTCCAGACTCTTCCCTCCCTCCCTCC CAGA-P
X'-Y'	TCCCTCCCTCCCTCCCAGTCCAGACTCTAACTATACAACCTA CTACCTCAA-P
EAD2 (Y)	CTGGGAGGGAGGGAGGGA
cEAD2	TCCCTCCCTCCCTCCCAG
target X	TGAGGTAGTAGGTTGTATAGTT
Random	GACTGTTTATAGCTGTTGGAAG
M2	TGAGGTAGTAGGTTGTATA*CC*T
M3	TGAGGTAGTAGGTTGTAT*TCA*T

Table S2 Comparison of different homogeneous electrochemical assays for DNA detection

Analytical method	Reporter	Dynamic range	The limit of detection
Strand displacement polymerization[1]	Ferrocene-PNA	0.1 nM-1 nM	100 pM
ExoⅢ-aided target recycling (label-free)[2]	Intercalated $[Os(bpy)_2(dppz)]^{2+}$	2.5 nM-100 nM	2.5 nM
Active-inactive switching molecular beacon[3]	Carminic acid labeled beacon	60 nM-1.4 μM	30 nM
ExoⅢ-assisted target recycling[4]	MB labeled beacon	20 pM-300 pM	20 pM
Polymerase Chain reaction[5]	DNAzyme catalyzed precipitation	0.1 nM-100 nM	100 pM
Reporter-EXPAR (this work)	G-quadruplex	1 pM-1 nM	1 pM

Table S3 DNA detection spiked with 20% human serum via X′-Y′/ Y′-Y′ strategy (n=3).

$C_{(DNA)}$	Δi (Without spiking)	Δi (Spiked with 20% human serum)	Recovery
100 pM	1.21 nA (±0.030)	1.36 nA (±0.128)	112%
1 nM	1.69 nA (±0.049)	1.62 nA (±0.178)	95.9%

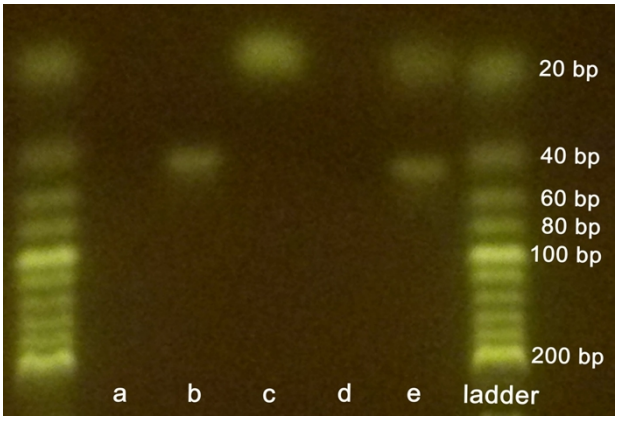

Figure S1 Agarose gel electrophoresis analysis of the production of R-EXPAR initiated with (land b, e) or without (land a, d) 10 nM EAD2. As EAD2 (a 18 nt length single-stranded DNA) could not be sensitively discriminated by eyes (too short to dye well), a complementary sequence cEAD2 was added to form double-stranded (ds)DNA with EAD2. Land c was prepared with 1 μM EAD2/cEAD2 dsDNA; land d, e were land a, b in the presence of 1 μM cEAD2. In land e, one piece of dsDNA band was appearing remarkably above 20 bp band of ladder.

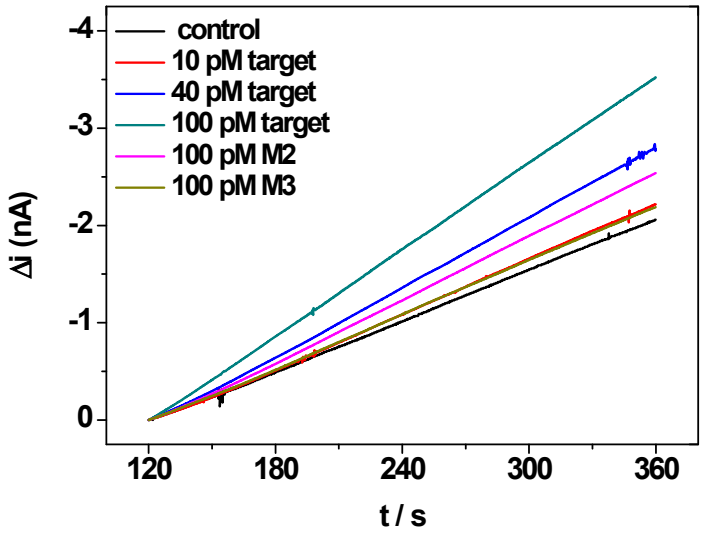

Figure S2 The selectivity of X'-Y'/Y′-Y′ strategy. Amperometric response curves of 1 mM HQ and 1 mM H_2O_2 with 12.5 μM hemin at an applied potential of -0.1 V in the presence of X'-Y'/Y′-Y′ samples: without target X (Negative control), 10 pM, 40 pM, 100 pM target X, 100 pM M2 and 100 pM M3, individually.

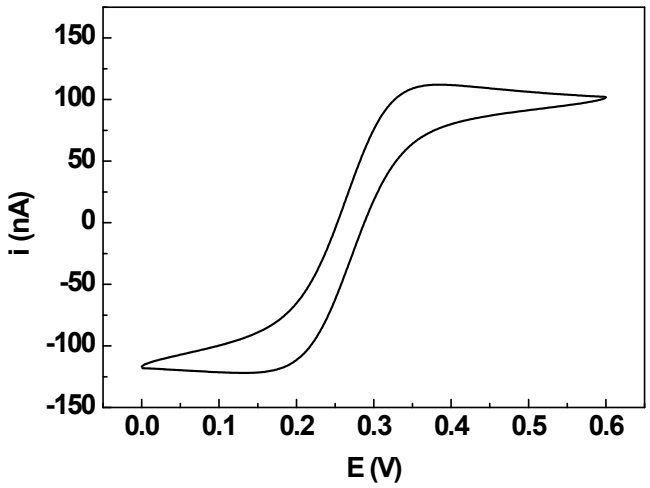

Figure S3. The CV of carbon fiber ultramicroelectrode inserted in the micropipette with 20 μL solution containing 5 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$, scan rate: 0.1V/s.

According to the formula[6]: $i_{ss} = 2\,nFAD_0C_0^*/r_0 \ln\tau$

$i_{ss} = 1.02\times10^{-7}$A

n=1

F= 96485 C/mol

$D_0 = 0.65\times10^{-5}\ cm^2/s = 6.5\times10^{-10}\ m^2/s$

$C_0^* = 5\times10^{-3}$mol/L =5 mol/m^3

$r_0 = 3.5\times10^{-6}$ m

τ=3 s lnτ= 1.10

The surface area of carbon fiber ultramicroelectrode was calculated to be $A = 6.25\times10^{-10}\ m^2$.

References

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