Supplementary Information (SI) for Analyst.
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Supporting information

Reliable and Precise Lipoprotein Detection based on the Self-Priming Hairpin-Triggered Cas12a/crRNA based Signaling Strategy

Experimental section

1. Materials and reagents

The antibody used in this research, including the anti-ApoA1 rabbit polyclonal antibody and anti-ApoB rabbit polyclonal antibody (Ab) were purchased from Thermo Fisher Technology (China) Co., LTD. (Beijing, China). All oligonucleotides for the signal amplification process were purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China). The Klenow fragment (3'-5' exo-) DNA polymerase, Nb.BbvCI, 10× NEBuffer™ 2 (containing 100 mM Tris-HCl, 500 mM NaCl, 100 mM $MgCl_2$, and 10 mM DTT, pH 7.9), and 10× CutSmart® buffer (containing 200 mM Tris-Ac, 500 mM KAc, 100 mM $Mg(Ac)_2$, and 1 mg/ml BSA, pH 7.9) were acquired from New England Biolabs Inc. (Beverly, MA, USA). Furthermore, the fluorescence spectra were captured using a Hitachi fluorospectro photometer. All solutions were made using Milli-Q integral purified deionized water.

2. Lipoprotein isolation

Human serum samples were collected from volunteers at Chongqing university Fuling hospital. The gradient density ultracentrifugation method was employed to separate lipoprotein classes (LDL and HDL). The centrifugations were conducted at a speed of 70,000 rpm at 4 ℃: the initial procedure involved placing 2 mL of a 0.195 M NaCl solution, with a density (ρ) of 1.006 g/mL, on top of 4 mL of human serum in a centrifuge tube. After 8 h of centrifugation, the top layer included the fraction of very low-density lipoprotein (vLDL). The lowermost stratum comprising LDL, HDL, and other serum proteins was transferred to a fresh centrifuge tube, and then overlaid with 2 mL of a solution containing 0.195 M NaCl and 2.44 M NaBr (with a density of 1.063 g/mL). Following a 10-hour centrifugation, the LDL fraction was able to be extracted from the uppermost layer. The lower layer, which consisted of HDL and other serum proteins, was transferred to a separate tube containing a solution composed of 0.195 M NaCl and 7.65 M NaBr (with a density of 1.478 g/mL) in a volume of 2 mL. Following a 16-hour period of mixing and centrifugation, the uppermost layer containing High-Density Lipoprotein (HDL) was gathered.

3. Preparation of "a" sequence with the cholesterol binding protein.

The conjugation of a sequence with the cholesterol binding protein relies on the robust interaction between streptavidin and biotin. Initially, 100 μL of cholesterol binding protein (1 mg/mL) was mixed with Sulfo-NHS-LC-Biotin (20 nmol) and the mixture was incubated for 2 h at room temperature. The mixture was then ultrafiltrated using 30 kDa centrifugal filter units for 3 times. Subsequently, 100 μL of streptavidin (1

mg/Ml) was added to the mixture, and the mixture was agitated for 1 h. A 5 µL of a solution containing 0.1 mg/mL of biotin-conjugated cholesterol binding protein was then added to the mixture and incubated at room temperature for 2 h. Afterwards, the mixtures purified using centrifugal filter units for 3 times.

4. Procedures for the LDL detection

Before the assay, 1 µM of H1 probe and 1 µM of H2 probe in 0.5× NEBuffer™ 2 and 0.5× CutSmart® buffer were subjected to heating at 95 ˚C for 5 min and then cooled down slowly to 25 ˚C (at a rate of 0.1 ˚C per second) in order to create the stable hairpin structure independently. The various concentrations of the LDL solution were diluted to 50 µL and were added to the ApoB antibody on a plate at room temperature for 45 min. The liquid portion above the sediment was subsequently extracted, resulting in the presence of the LDL-ApoB-antibody@plate. Next, 10 µL of the complex of “a”-cholesterol binding protein was added to the plate and left to incubate at room temperature for 45 min. The liquid supernatant was subsequently extracted, resulting in the “a”-LDL-ApoB-antibody@plate. The assay solution was generated by combining 50 µL of solution containing 500 nM of H1 probe, 500 nM of H2 probe, 250 µM dNTPs, 1 U/µL DNA polymerase, 0.375 U/µL Nb.BbvCI, 0.5× NEBuffer™ 2, and 0.5× CutSmart® buffer. The solution was then incubated at 37 °C for 30 min. After incubation, 2 µL of Cas12a and 2 µL of crRNA sequence were added. Following a 20-min incubation at 25 °C, the fluorescence emission spectra were recorded. The first spectrum was measured in the range of 500-600 nm with an excitation wavelength of 485 nm for FAM. The second spectrum was obtained in the range of 550-650 nm with an excitation wavelength of 534 nm for Cy3.

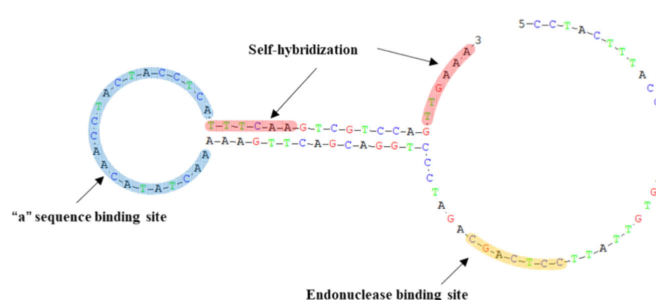

Figure S1. Schematic illustration of the functional sections of the H1 probe.

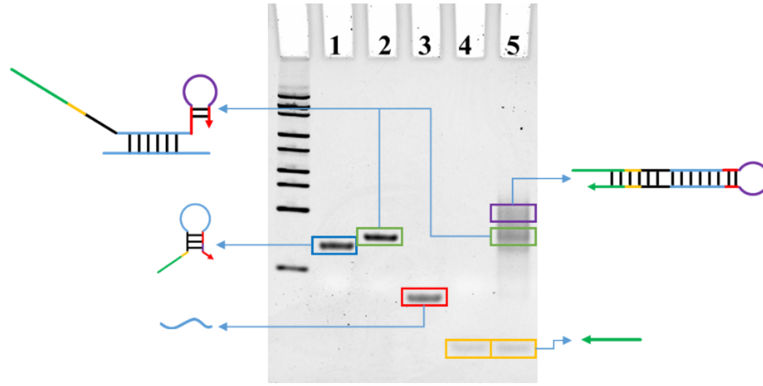

Figure S2. Gel electrophoresis result of the "a" sequence mediated self-priming and DNA polymerase assisted chain extension process. 1, H1 probe; 2, H1 probe+ "a"; 3, "a" sequence; 4, "e"; 5, H1 probe+ "a"+ DNA polymerase+ endonuclease.

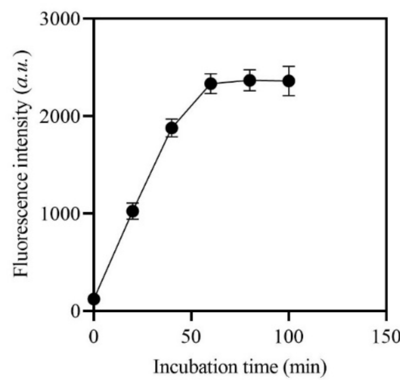

Figure S3. Fluorescence intensities of the approach when detecting LDL with different incubation time.

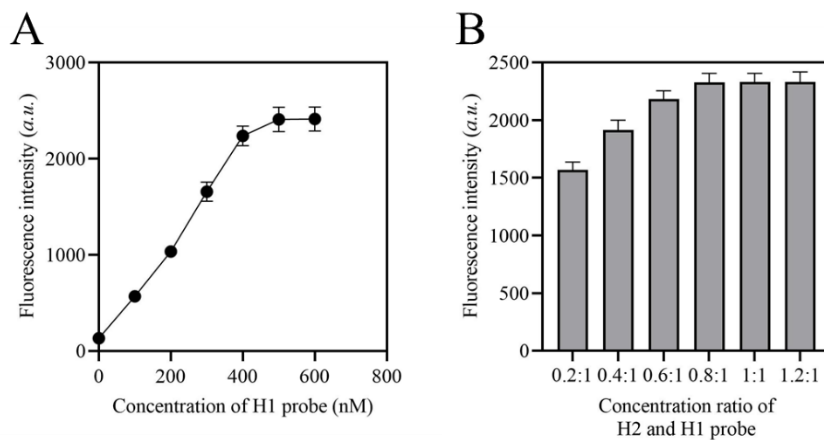

Figure S4. Fluorescence intensities of the approach when detecting LDL with different concentrations of H1 probe (A) and different concentration ratios of the H2 probe and H1 probe.

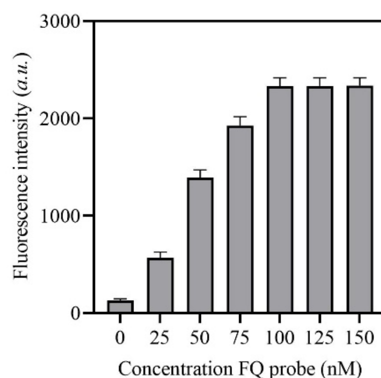

Figure S5. Fluorescence intensities of the approach when detecting LDL with different concentration of FQ probe.

Table S1. Oligonucleotide sequences used in this research

Title	Sequences (5' to 3')
"a"	TGA GGT AGT AGG TTG TAT AGT T
H1 probe	C CTA CTT TAC CAG ACA GTG TTA TTC CTC AGC AGA TCC CTG GAC GAC TTG AAA AAC TAT ACA ACC TAC TAC CTC A TTT CAA GTC GTC CAG TTG AAA
H2 probe	TAG CTT ATT TAC GTC GCC GTC CAG CTC GGA TCC CTG GAC GAC TTG AAA CCA TCT TTA CCA GAC AGT GTT A TTT CAA GTC GTC CAG TTG AAA
crRNA	UAA UUU CUA CUA AGU GUA GAU UCA ACA UCA UCU GAU AAG C

Table S1. A brief comparison of the proposed method with former ones.

Title	Signal amplification strategy	Recognizing targets	Sensitivity (LDL)	Advantages and disadvantages	Ref
The method	Self-priming initiated chain extension+ CHA	ApoB protein; cholesterol	5.4 mg/dL	Advantages: high selectivity; anti-interferences capability; high sensitivity; Disadvantages: could be loaded on portable devices.	
ALBK	Enzyme catalytic reaction	cholesterol	> 100 mg/dL	Advantages: mature application; Disadvantages: low sensitivity; may be interfered by the free cholesterol; low selectivity.	
ELISA	Enzyme catalytic reaction	ApoB (for LDL)	> 80 mg/dL	Advantages: mature application; high repeatability Disadvantages: low sensitivity; may be interfered by the free ApoB protein; low selectivity.	
Accurate method	RCA+ proximity ligation	ApoB protein; cholesterol	7.3 mg/dL	Advantages: high selectivity; anti-interferences capability; Disadvantages: low sensitivity; multiple enzymes are required for signal amplification.	[1]
Efficient platform	HCR-DNAzyme	ApoB protein; cholesterol	30 mg/dL	Advantages: high selectivity; anti-interferences capability; Disadvantages: low sensitivity; complicated primer design.	[2]

Notes: ALBK, Abell−Levy−Brodie−Kendall; CHA, catalytic hairpin assembly; RCA, rolling circle amplification; ELISA, enzyme-linked immunosorbent assay. HCR, hybridization chain reaction.

References:

1. Zhang, X.; Li, J.; Yang, M.; Huang, H.; Wang, H.; Zhang, H., Accurate and sensitive low-density lipoprotein (LDL) detection based on the proximity ligation assisted rolling circle amplification (RCA). *Anal Methods* **2024,** *16* (13), 1894-1900.
2. Chen, H. J.; Hu, Y.; Yao, P.; Ning, D.; Zhang, Y. P.; Wang, Z. G.; Liu, S. L.; Pang, D. W., Accurate and Efficient Lipoprotein Detection Based on the HCR-DNAzyme Platform. *Anal. Chem.* **2021,** *93* (15), 6128-6134.