Electronic Supplementary Material (ESI) for Analyst.

A novel steric effect-regulated isothermal exponential amplification technology for one-step homogeneous sensing of proteins

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Experimental

Materials. All oligonucleotides of HPLC purity were synthesized from Sangon Biotech Co., Ltd. (Shanghai, China). Sequences of these oligonucleotides are listed in Table S1. Bst 2.0 WarmStart DNA polymerase (8000 units mL^{-1}), Nt.BstNBI nicking endonuclease (10000 units mL^{-1}), deoxyribonucleotide triphosphates (dNTPs, 10 mM) and 10× NEBuffer 3.1 were purchased from New England Biolabs (Ipswich, MA). Anti-Digoxin mouse monoclonal antibody (IgG, 0.5 mg mL^{-1}) was purchased from GeneTex, Inc. (Irvine, CA, USA). Avidin from egg white (Bio-Ultra), Elastase from porcine pancreas and human thrombin were purchased from Sigma Aldrich (St. Louis, MO, USA). SYBR Green I (10,000×) was purchased from Invitrogen Life Technologies (Carlsbad, CA, USA). DNA-grade water (DNase- and Protease-free) was purchased from Fisher Scientific (Pittsburgh, PA, USA). The clinical serum samples of healthy person were obtained from the Hospital of Zhejiang University. All solutions were freshly prepared with DNA-grade water.

General Procedure of SER-EXPAR. First, a stock solution of 20 nM EBH, 50 nM EXPAR template, 2 U Nt.BstNBI, 0.8 U Bst 2.0 WarmStart DNA polymerase, 0.4 mM dNTPs, 0.5× SYBR Green I, 1× NEBuffer 3.1 (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 μg/ml BSA) and water was freshly prepared on ice. The final reaction mixture was obtained by distributing 22.5 μL stock solution and 2.5 μL target protein of varying concentrations into 8-strip PCR tubes. After vortexing and centrifugation, these PCR tubes were immediately transferred onto a Bio-rad CFX-96 real-time PCR system and incubated at 55 °C. The fluorescence of SYBR Green I was measured at 1 min intervals.

Gel Electrophoresis. In order to demonstrate the feasibility of SER-EXPAR, 15% non-denaturing polyacrylamide gel electrophoresis (PAGE) was used to characterize the interactions among avidin, Nt.BstNBI and biotin-labeled EBH. Specifically, a solution containing 1 μM EBH_6 and 1 μM Nt.BstNBI or 1 μM target protein (or both) in 1× NEBuffer 3.1 (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 μg/ml BSA, pH 7.9@25 °C) was first incubated at 55 °C for 15-20 min. Afterwards, 5 μL of each resultant were loaded onto 15% non-denaturing polyacrylamide gel and subjected to 150 V constant voltage in 0.5× TBE buffer (45 mM Tris, 45 mM borate, 1 mM EDTA, pH 8.3) for 60 min. The gel was then stained by 1 × SYBR Green I and analyzed on a Maestro Ex IN-VIVO Imaging System (CRI).

Sensing of Avidin in Human Serum. In order to demonstrate the utility of SER-EXPAR in complex biological matrix, it was performed in the presence of human serum. The serum was used, without dilution or other pretreatment,

to dilute target protein (avidin) by tenfold in the series. Subsequently, 2.5 μL of spiked serum was introduced into SER-EXPAR system and subjected to the same procedure as described above. The results were compared with those obtained in buffer.

Table S1. Sequences of the Oligonucleotides.[a]

Name	Sequence (5′-3′)
EXPAR template	CTCACGCTACGGACGACTCTCTCACGCTAC
EBH_1	CATCACTGCACTGACTCACGTTTTTCGTGAGTCAACTCCTGTAAG
EBH_2	CATCACTGCACTGACTCACGTTTTTCGTGAGTCAACT
EBH_3	CACTGACTCACGTTTTTCGTGAGTCAACTCCTGTAAG
EBH_4	CACTGACTCACGTTTTTCGTGAGTCAACT
EBH_5	CATCACTGCACTGACTCACGTTTTTCGTGAG (biotin-T) CAACTCCTGTAAG
EBH_6	CATCACTGCACTGACTCACGTTTTTCG (biotin-T) GAGTCAACTCCTGTAAG
EBH_7	CATCACTGCACTGACTCACGTTTT (biotin-T) CGTGAGTCAACTCCTGTAAG

[a]The underlined bases represent the basic sequences of hairpin probes' stem-loop structure, among which the red bases are the recognition sites of Nt.BstNBI.

Table S2. Comparison of different methods based on small molecule-linked DNA for the detection of avidin.

Method	Detection limit	Detection range	Time	Complexity	Ref.
Small molecule-linked loop DNA probe	0.4 nM	0 nM～200 nM	～30 min	Multi-steps	1
Modular, DNA-Based Beacon	low nanomolar	1 nM～10 nM	10 min	One-step, need surface modification	2
Fluorescent copper nanoparticles	0.1 nM	0.5 nM～100 nM	>1.5 h	Multi-steps	3
Sterically and allosterically tunable HCR	1.07 nM	4 nM～1 μM	3 h	Multi-steps	4
Strand displacement competition reaction	nanomolar	0 nM～2 nM	overnight	One-step	5
This work	1.29 nM	5 nM～50 nM	20-25 min	One-step	/

References:

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4 F. F. Ban, H. Shi, C. Feng, X. X. Mao, Y. M. Yin and X. L. Zhu, *Biosens. Bioelectron.,* 2016, **86**, 219-224.

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Supplement Figures

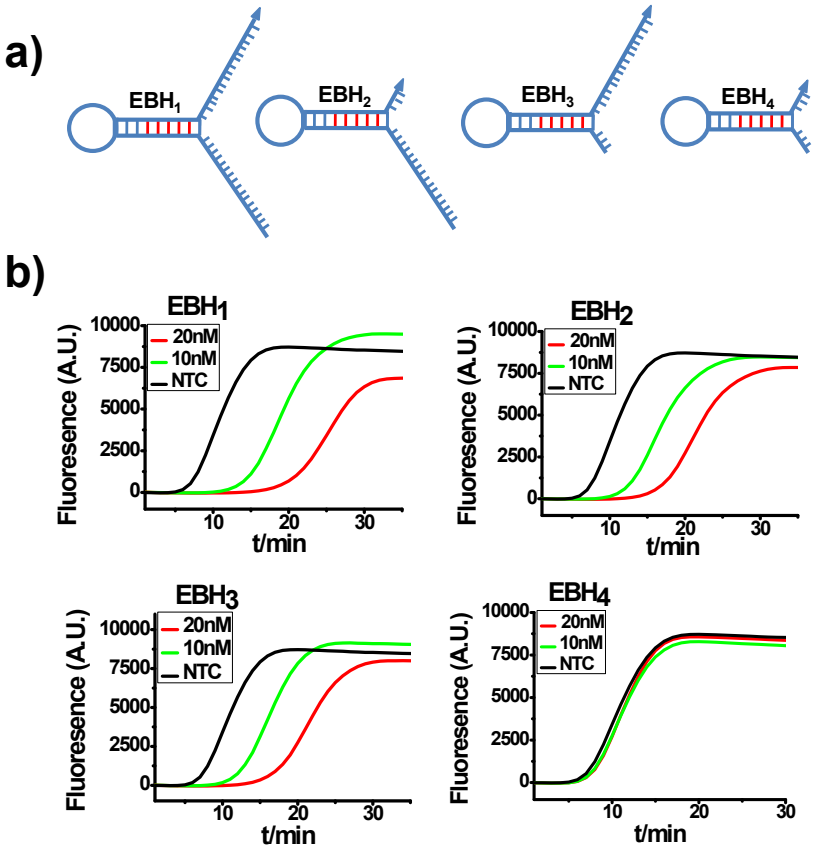

Figure S1. (a) Four kinds of EBH with different side arm length. (b) Effects of 10nM and 20nM EBH_1～EBH_4 on the real-time fluorescence of EXPAR; NTC represents no-EBH control.

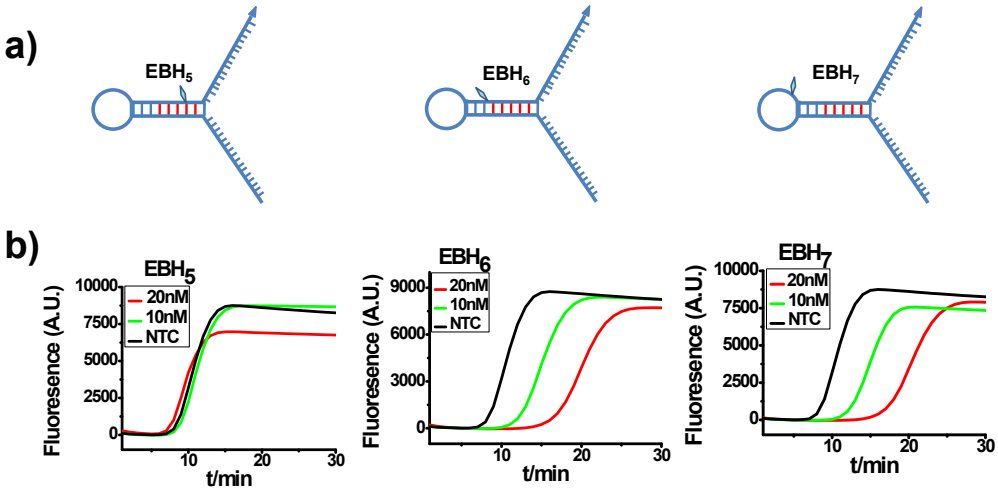

Figure S2. (a) Three kinds of EBH labeled with biotin at different sites, *i.e.*, among, near and relatively far from the recognition sequence of Nt.BstNBI. (b) Effects of 10nM and 20nM EBH_5～EBH_7 on the real-time fluorescence of EXPAR; NTC represents no-EBH control.

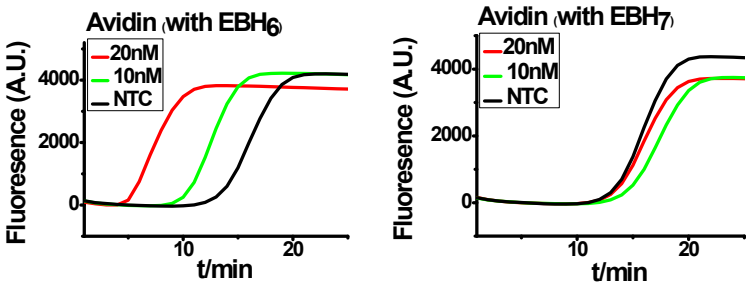

Figure S3. Real-time fluorescence curves caused by 10nM and 20nM avidin in the presence of 20nM EBH_6 (Left) and EBH_7 (Right); NTC represents no-avidin control.

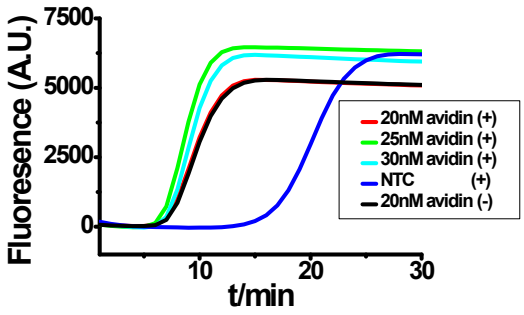

Figure S4. Real-time fluorescence curves caused by 20nM, 25nM and 30nM avidin; NTC represents no-avidin control; "+" represents that the system is in the presence of 20nM EBH_6 and "-" represents that the system is in the absence of EBH_6.

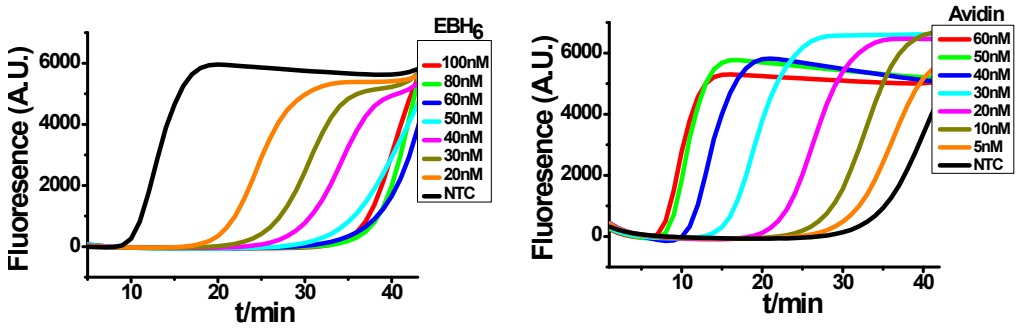

Figure S5. (a) Real-time fluorescence curves caused by various concentrations of EBH_6 without avidin; NTC represents no-EBH_6 control. (b) Real-time fluorescence curves caused by various concentrations of avidin in the presence of 50nM EBH_6; NTC represents no-avidin control.

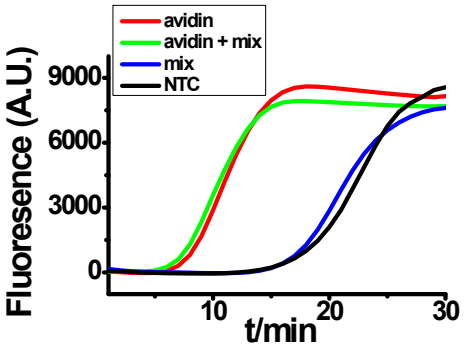

Figure S6. Real-time fluorescence curves caused by 20 nM avidin and the protein mixture in the presence of 20 nM EBH_6. Mix represents the mixture of 200 nM IgG, 200 nM thrombin and 200 nM elastase. NTC represents no-protein control. (Overmuch proteins (*i.e.* 3*500 nM) in the buffer would influence the rate of EXPAR, thus the total amount of proteins in the mix was set 3*200 nM that had little effect on the EXPAR).