Supporting Information

Programmable Mg^{2+}-dependent DNAzyme switch by the catalytic hairpin DNA assembly for dual-signal amplification toward homogenous analysis of protein and DNA

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Experimental Section.

Materials and chemicals. Exonuclease I was purchased from New England Biolabs Ltd. (Ipswich, MA, USA). Avidin, bovine serum albumin (BSA), human IgG and fetal bovine serum were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). Human thrombin was supplied by Dingguo Biotech Co., Ltd. (Beijing, China). Acrylamide/bisacrylamide 39:1 40% gel stock solution, N,N,N',N'tetramethylethylenediamine (TEMED) and Ammonium persulfate (APS) was purchased from Yantai Science and Biotechnology CO., Ltd. (Yantai, China). T safe^{TM} Nucleic Acid Gel Stain was purchase from Tanon Science & Technology Co., Ltd. (Shanghai, China) The HPLC-purified oligonucleotide sequences were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China) and listed in Table S1. All other chemicals were obtained from Shanghai Chemical Reagents (Shanghai, China) and used without further purification.

Protein and target DNA detection by using the programmable Mg^{2+}-dependent DNAzyme switch by the catalytic hairpin DNA assembly. 20 μL of 2μM biotin-linked DNA probe in 1× NEB buffer I (10 mM Bis Tris Propane-HCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.0@25^{o}C) was added into 30 μL of the sample solution containing different concentrations of avidin (final avidin concentrations ranging from 0 to 10 nM). The mixture was incubated at room temperature for 30 min to allow complete interaction between avidin and biotin. Then, 10 U Exo I was added in the mixture and incubated for 30 min at 37 ^{o}C to perform the digestion reaction, followed by inactivation of Exo I at 80 ^{o}C for 20 min. After cooling to room temperature, the resulting solution was incubated with a constant concentration of HP1 and HP2 (final concentration of HP1 and HP2 0.2 μM) in 1× NEB buffer II (10 mM Tris-HCl,50 mM NaCl, 10 mM $MgCl_2$, 1 mM DTT, pH 7.9@25^{o}C) at room temperature for 3 hr. subsequently, the ribonucleobase (rA) containing substrate (0.25 μM) and $MgCl_2$ (20 mM) were added to the sample. After incubation at room temperature for 2 hr, the fluorescence responses were recorded.

For the detection of target DNA, different concentrations of target was annealed

with a constant concentration of HP3 (final concentration 100 nM) before the addition of other components.

Polyacrylamide Gel Electrophoresis. To prepare the hydrogel, 4 mL 40% gel solution (39:1), 160 μl 50×TAE Buffer, 80 μl 10% APS, 4 μl TEMED and 3.76 ml deionized water were mixed. This mixture contained a final gel percentage of 20%. The polyacrylamide gel electrophoresis (PAGE) was carried out in 1×TAE buffer at a constant voltage of 165 V for about 90 min at room temperature, and then stained in T safe^{TM} dye solution.

Instruments. Fluorescence measurements were performed using a Hitachi F-4600 spectrofluorimeter with a scan rate at 1200 nm/min (Tokyo, Japan). The excitation wavelength was set to 490 nm and the 24 photomultiplier voltage was 700V. The slits for excitation and emission were set at 5 nm / 5 nm.

Tab. S1 Sequence of synthesized oligonucleotide probes for protein and target DNA detection[a]

Name	Sequence (5' to 3')
Biotin-linked ssDNA	TTACTATTACTATCTCTAAGATCGAA-biotin
HP1	GATATCAGCGATCTTAGAGATAGTAATAGTAAAAGCACCCATGTTACT CTTTACTATTACTATCTCT
HP2	TAATAGTAAAGAGTAACATGGGTGCTTTTACTATTACTATCTCTAAGC ACCCATGTTACTCT
HP3	TTACTATTACTATCTCTAAGATCGAGAGTCTTCCAGTGTGATGATCGAT C TT
DNAzyme substrate	FAM-AGAGTATrAGGATATC-BHQ
S1	GATATCAGCGATCTTAGAGATAGTAATAGTAAA
S2	TTTACTATTACTATCTCTAAGCACCCATGTTACTCT
TD	TCATCACACTGGAAGACTC
1MT	TCATCAAACTGGAAGACTC
NC	ACGTTGCATATCGACTAGC

[a]HP denotes hairpin DNA probe. S1 and S2 are the directly synthesized ssDNA sequences containing the Mg^{2+}-dependent DNAzyme subunits, respectively. TD, 1MT, and NC denote target DNA related p53 gene, single-base mismatched DNA, and non-complementary DNA, respectively. The underlined letter in 1MT indicates the mismatched base.

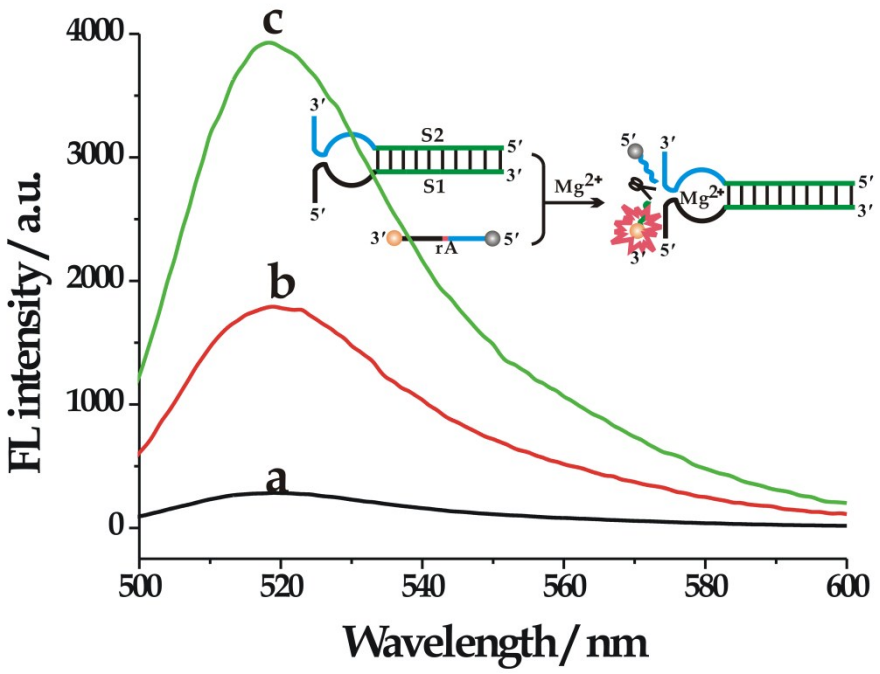

Fig.S1 The fluorescence emission spectra for the directly synthesized Mg^{2+}-dependent DNAzyme by the hybridization of S1 and S2 toward substrate. The Mg^{2+}-dependent DNAzyme was prepared by using the same concentration of S1 and S2 sequence. The curves for (a), (b) and (c) were for 0, 1 and 200 nM synthesized Mg^{2+}-dependent DNAzyme, respectively. The substrate concentration was 250 nM. The inset shows the scheme for the synthesized Mg^{2+}-dependent DNAzyme formed by S1 and S2.

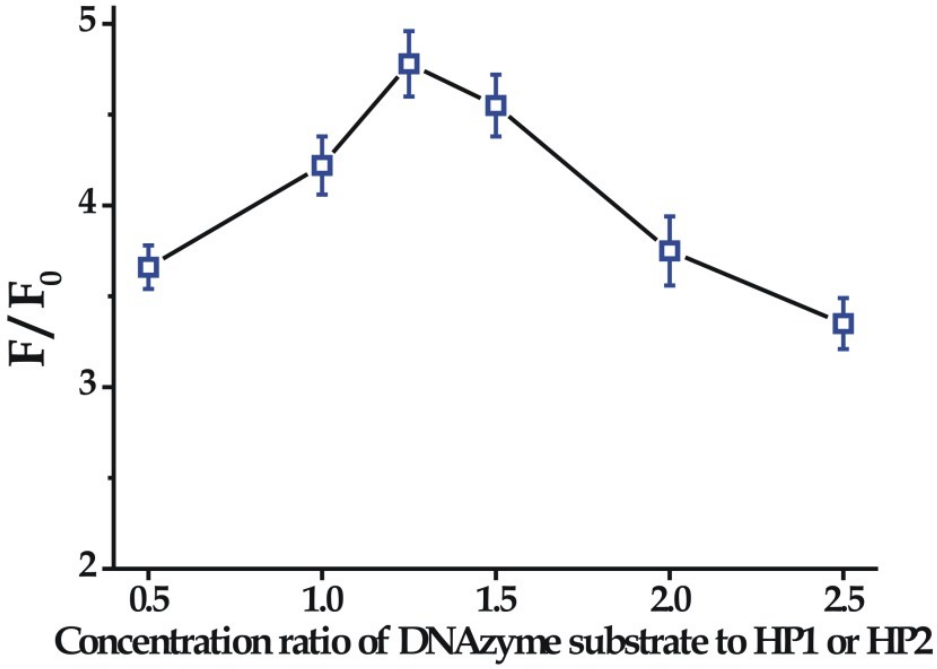

Fig.S2 The optimization for the concentration ratio of DNAzyme substrate to HP1 or HP2. The HP1 was the same concentration with the HP2. The F and F_0 represent the fluorescence response in the presence and absence of 10 nM avidin, respectively.

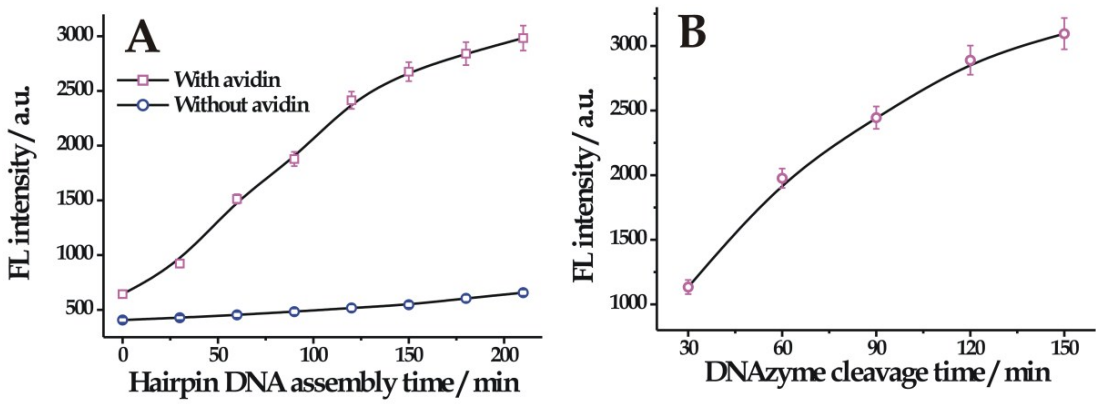

Fig. S3 (A) Time optimization for hairpin DNA assembly. (B) Time optimization for DNAzyme cleavage.

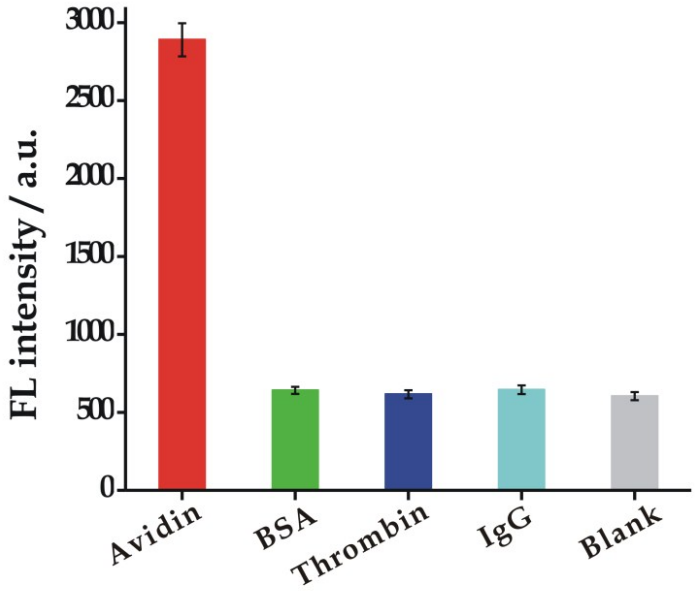

Fig.S4 Selectivity of the fabricated sensing system to avidin, bovine serum albumin (BSA), thrombin and immunoglobulin G (IgG) at 10 nM, respectively. Error bars are obtained based on three independent measurements.

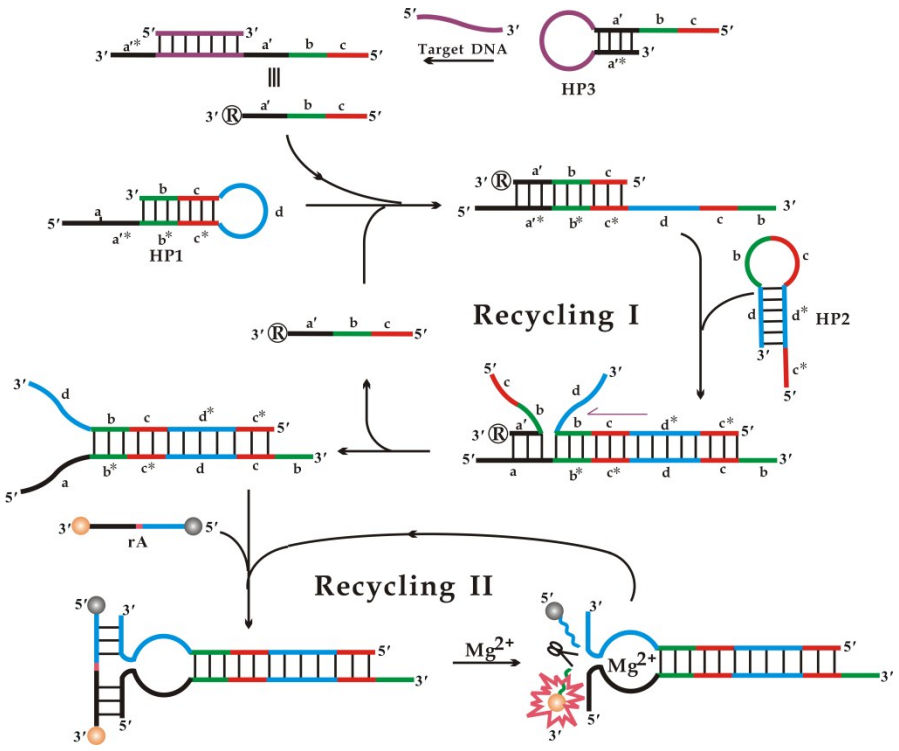

Fig.S5 Schematic illustration for the target DNA detection based on the catalytic hairpin DNA assembly-programmed Mg^{2+}-dependent DNAzyme switch.

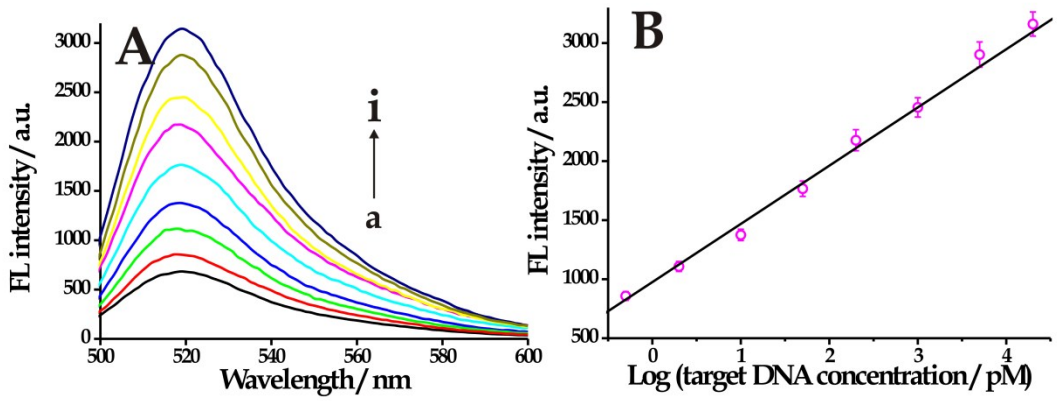

Fig.S6 (A) Fluorescence responses corresponding to the analysis of different concentrations of target DNA. The concentrations of target DNA for the curves (a) to (i) are: (a) 0 M, (b) 0.5 pM, (c) 2 pM, (d) 10 pM, (e) 50 pM, (f) 200 pM, (g) 1 nM, (h) 5 nM, (i) 20 nM. (B) The linear relationship between the fluorescence intensity and the logarithm value of the target DNA concentration. Error bars represent standard deviations of measurements (n = 3).

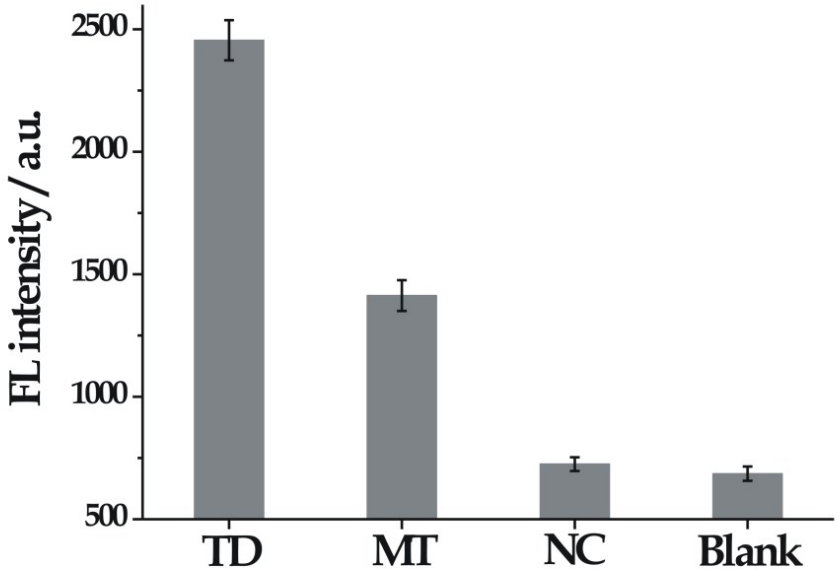

Fig.S7 The bar chart of the fluorescence intensity toward the blank and three various DNA sequences including complementary target DNA (TD), single-base mismatched DNA (MT), and non-complementary DNA (NC). The concentrations of various DNA sequences were all 1 nM. Error bars represent standard deviations of measurements (n=3).

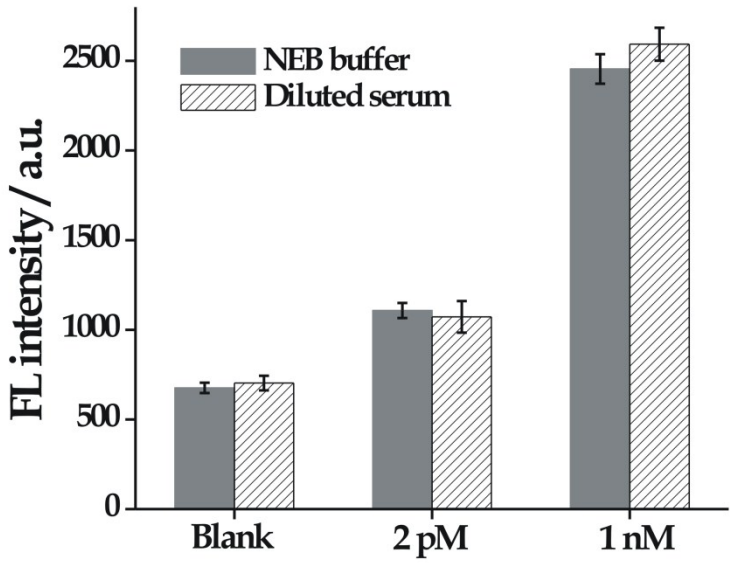

Fig.S8 Fluorescence intensity obtained for the developed sensing system in buffer and 2% fetal bovine serum (50 fold diluted) spiked with two different target DNA concentrations. The error bars represent the standard deviation of three measurements.

Tab.S2 Comparison of detection performance for target DNA by ours and those reported methods

Method	Detection limit	Strategy	Ref.
Fluorescence	20 pM	DNAzyme-based amplification	[1]
Fluorescence	10 pM	Autonomous Ligation DNAzyme Machinery	[2]
Fluorescence	1 pM	Catabolic DNAzyme-mediated process	[3]
Fluorescence	10 fM	Assembled DNAzyme wires	[4]
Fluorescence	10 pM	Catalytic hairpin assembly and bimolecular beacons	[5]
Fluorescence	20 fM	Exo III-aided cascade target recycling and DNAzyme	[6]
Fluorescence	10 pM	Zn^{2+}-ligation DNAzyme-driven DNAzyme cascade	[7]
Fluorescence	80 pM	Catalytic hairpin assembly and SYBR Green I	[8]
Fluorescence	0.5 pM	Programmed Mg^{2+}- dependent DNAzyme by catalytic hairpin DNA assembly	This work

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