Supplementary Material

Target-switched artificial biochemical circuit for a versatile and sensitive colorimetric detection platform

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S1 Experimental section

S1.1 Materials and apparatus

Vent (exo-) DNA polymerase supplied with 10× ThermoPol buffer (20 mM Tris-HCl,10 mM KCl, 10 mM $(NH_4)_2SO_4$, 2 mM $Mg(SO_4)_2$, 0.1% Triton X-100, pH 8.8 @ 25 ºC), Nt.BstNBI supplied with 10× NEBuffer (50 mM Tris-HCl, 100 mM NaCl, 10 mM $MgCl_2$, 100 μg mL^{-1} BSA (pH 7.9 @ 25 ºC) and the deoxynucleotide triphosphates (dNTPs) mixture were obtained from New England Biolabs (Beijing, China). Dimethyl sulfoxide (DMSO), $CHCl_3$, and C_2H_5OH were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). H_2O_2 was purchased from Tianli Chemical Reagent Co., Ltd (Tianjin, China). Hemin, 2,2-azino-bis(3-ethylbenzothiozoline-6-sulfonic acid) ($ABTS^{2-}$) were purchased from Yuanye Bio-Technology Co., Ltd

(Shanghai, China). The hemin stock solution (10 μM) was dissolved in DMSO and stored at -20 ºC for further use. All oligonucleotides were synthesized and HPLC-purified by Sangon Biological Engineering and Technology & Services Co., Ltd. (Shanghai, China). The sequences were listed in Table S1.

Table S1 Sequences of the synthetic oligonucleotides

Nucleic Acid ID	Sequence (5'-3')
target DNA	CGT AAG TCT CCG AGG TTG CCA TCG ATG ATT TGA ACT
MH	**GAT GGC AAC CTC GGA GAC TTA CGG** AGG GAC TCG CGT AGG TAC TAA CGT AAG TCT CCG ATT ACC TAC GC
template	AAT GCC CAA CCC GCC CTA CCC ACG AGA CTG ACT CCG ATG GCA ACC TCG GAG ACT TAC GGA CTG ACT CCG ATG GCA ACC TCG GAG ACT TAC G
m2-DNA	CGT AAG TCT CCG ACG TTC CCA TCG ATG ATT TGA ACT
m3-DNA	CGT TAG TCT CCG ACG TTC CCA TCG ATG ATT TGA ACT
non-cDNA	GAT GAA GTT CAA ATC ATC GAT GGC AAC CTC GGA GAC

The bold sequence is the target-specific recognition domain. The underlined bases are the antisense sequences of specific fragments for Nt.BstNBI recognition. The sequences marked in the same color are complementary sequences. The mismatched positions in m2-DNA and m3-DNA are marked in red.

The preparation of hairpin probes was accomplished with a GeneAmp® PCR system 9700 (Applied Biosystems, USA). The UV-Vis absorption spectra were collected with a UV-2600 spectrophotometer (Shimadzu, Japan) at room temperature in the wavelength range of 390 nm to 500 nm. The RGB values were analyzed by the Color Recognizer APP (Xiyi Technology) loaded from the APP store.

S1.2 Self-primed cascading isothermal EXPAR

Prior to use, the MH oligonucleotides were annealed to form hairpin structures. The self-primed EXPAR was accomplished by separate preparation of solutions A and B on an ice bath followed by mixing and incubating both solutions. 10 μL of solution A consisted of 200 nM MH, 500 μM dNTPs, 0.5× NEBuffer, and targets at various

concentrations. Solution B (10 μL) was composed of 0.8 U μL^{-1} Nt.BstNBI endonuclease, 0.2 U μL^{-1} Vent (exo-) DNA polymerase, and 300 nM template in 1× ThermoPol buffer. The same volume of solutions A and B were mixed to a total volume of 20 μL and incubated at 55 °C for 40 min. Finally, the resulting mixture containing EXPAR product was diluted to 80 μL for colorimetric analysis.

S1.3 The smartphone-integrated colorimetric measurement

The hemin/G DNAzyme was formed via the combination between hemin and the G-rich sequence from the EXPAR product by the addition of hemin (10 μM, 5 μL) into the EXPAR product (80 μL) and incubation at room temperature for 1 h. Next, the freshly prepared 25 μL of H_2O_2 (10 mM) and 25 μL of $ABTS^{2-}$ (20 mM) were added into the resulting solution, followed by incubation at room temperature for 15 min to accomplish the DNAzyme-catalyzed chromogenic reaction. The obtained solution was photographed with a smartphone, followed by the RGB values readout of the captured image by an installed APP. Then, the reaction solution was transferred to a quartz cell and placed in the spectrophotometer for measurement scanning from 390 to 500 nm. The UV-Vis absorption spectra showed a characteristic absorbance at 420 nm, which was employed to evaluate the target concentration-defined performance of our biosensor. In this way, the smartphone-integrated dual-readout colorimetric analysis of the target DNA was realized.

S1.4 Non-denaturing polyacrylamide gel electrophoresis (PAGE)

Different samples were loaded into the notches of the freshly prepared non-denaturing polyacrylamide gel (16%), and electrophoresis was performed at 100 V for

50 min in 1 × TBE buffer. After dying with gelred, the gel was transferred to a dark box where the electrophoresis image was taken using a digital camera under UV light.

S2 Results and discussion

The detailed principle of our proposed method

In this protocol, we proposed a novel colorimetric sensing platform based on the target-induced structure switch of a MH followed by self-primed cascade EXPAR to produce tremendous hemin/G DNAzyme for highly efficient signal amplification with the employment of spectrophotometer and smartphone as detection tools. The principle is demonstrated in Scheme 1. As shown in part A, MH was rationally designed with different domains including the hanging self-primer domain, the complementary sequence of self-primer and the nicking endonuclease (Nt.BstNBI)-specific recognition site in the loop, and the target recognition domain enclosing in the stem at its 5'-end. In the absence of the target DNA, MH is in an inactive configuration with its self-primer and a toehold sequence overhanging away from the stem as single-stranded DNAs (ssDNAs), leading to the EXPAR inhibition. Once the target DNA is present, it hybridizes with the target-specific recognition domain via the toehold-mediated strand displacement reaction, inducing the structure switch of MH to an activated configuration. In this state, the self-primer can hybridize with its complementary sequence to enable its extension along the MH track assisted by DNA polymerase, which results in the formation of a Nt.BstNBI-specific recognition sequence and the release of target to activate another MH. Meanwhile, the prolonged strand is cleaved

by Nt.BstNBI and extended again. Such cyclic extension-cleavage event realizes the release of numerous ssDNA amplicons. The released ssDNA amplicon acts as primer for another template to trigger the next EXPAR. As shown in part B, this template contains two repeated antisense sequences of the released ssDNA amplicon and a C-rich domain with two specific fragments for Nt.BstNBI recognition. The amplicon-triggered secondary EXPAR can produce more amplicons for recycling and a large number of G-rich sequences to combine with hemin, which results in the formation of hemin/G-quadruplex DNAzymes catalyzing the oxidation of colorless $ABTS^{2-}$ to green $ABTS^{\bullet-}$ with the presence of H_2O_2. The color change of the reaction solution is related to target concentration and respectively analyzed with a spectrophotometer and smartphone for dual quantification, followed by RGB analysis with the Color Recognizer APP (part C).

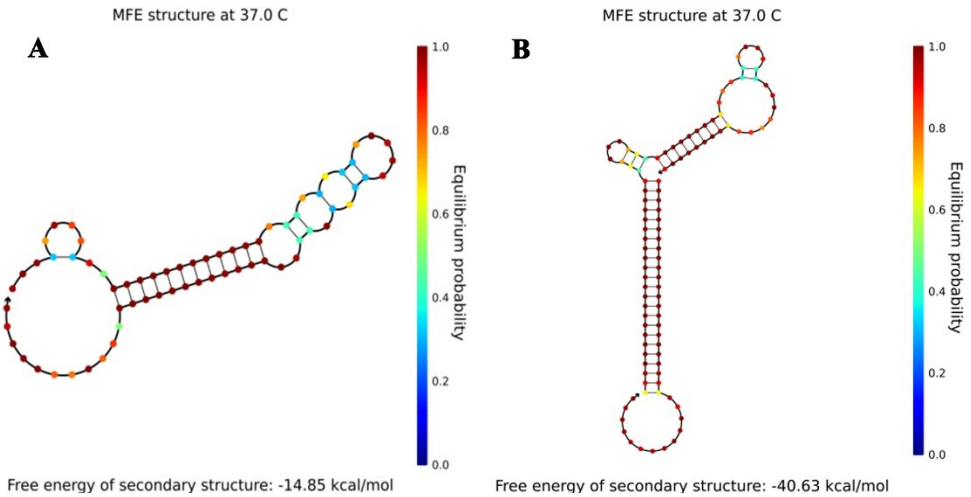

Fig. S1. Model of DNA secondary structures analysis. Minimum free energy (MFE) structure of (A) MH at 37 °C, (B) MH-target DNA interacting strands at a ratio 1:1 of MH and target DNA at 37 °C. The MFE structures were analyzed by NUPACK web server (http://www.nupack.org/). Copyright [Copyright © 2007-2021 Caltech. All rights reserved. http://www.nupack.org/home/terms].

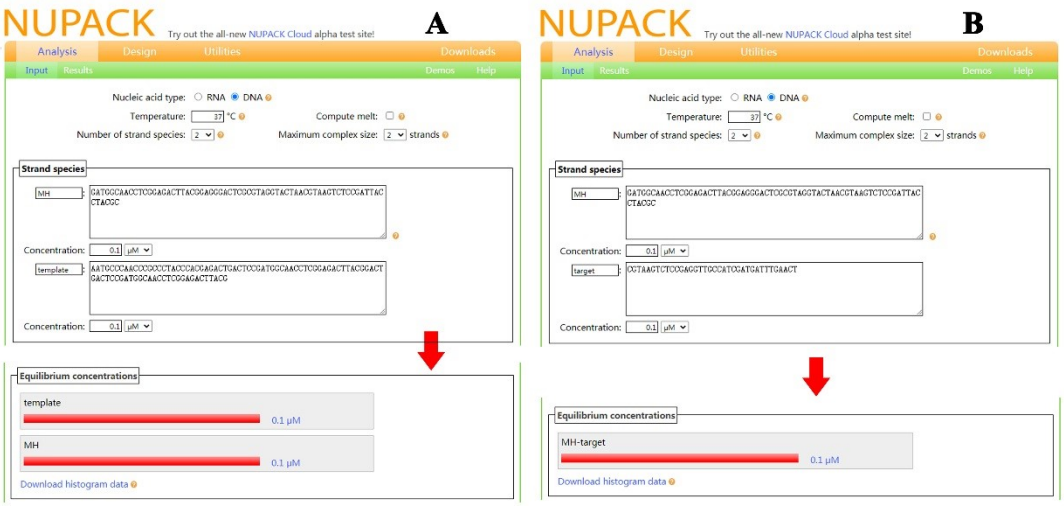

Fig. S2. Simulation of hybridization through NUPACK software (http://www.nupack.org/). (A) the input interface and output interface for simulating the hybridization between MH (100 nM) and another template (100 nM). (B) Input interface and output interface for simulating the hybridization of MH (100 nM) and target DNA (100 nM). Copyright [Copyright © 2007-2021 Caltech. All rights reserved. http://www.nupack.org/home/terms].

The stability of our proposed method

The stability of the biosensing platform was evaluated by continuous recording the UV-Vis spectra and G values of the images in the dual-readout detection step. As shown in Fig. S3, no obvious changes in the intensity of absorbance and G value were found during 10 min. After 10 min, the signal intensity respectively retained 90.2% and 111.3% of their initial responses. Significantly, the initial responses for UV-Vis and smartphone detection were recorded after 15 min of the DNAzyme-catalyzed chromogenic reaction. Such result indicated the acceptable stability of our proposed biosensing platform.

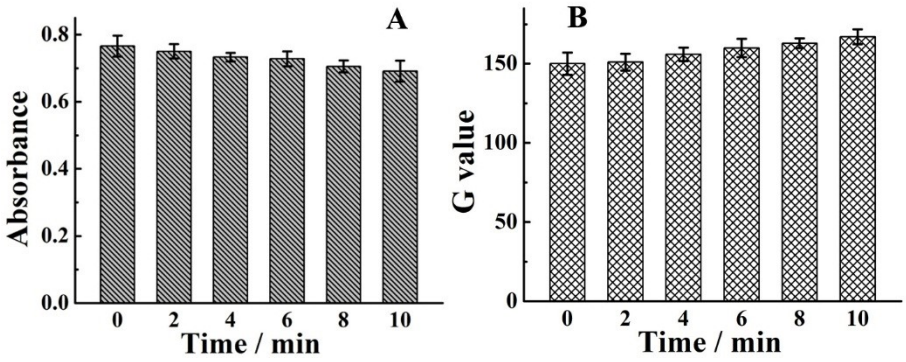

Fig. S3. (A) UV-Vis response intensities of this biosensor for the 5 pM target DNA detected every 2 min. (B) The corresponding respective G value of the images analyzed every 2 min.

Table S2 Analytical performance of the proposed method compared with the reported nucleic acid detection methods.

Detection method	Strategy	Linear range	LOD	Reference
Fluorescence	Quenching effect of Ti_3C_2 nanosheets on fluorescent dyes	0.5~50 nM	100 pM	1
Fluorescence	Isothermal helicase-dependent amplification	100 fM~10 nM	12.8 fM	2
Electrochemistry	CNT-AuNP amplification	0.1 pM~10 nM	5.2 fM	3
Electrochemistry	Based on gold-PET electrodes	54~340 nM	25 nM	4
Surface plasmon resonance	Dynamic and structural DNA nanodevice amplification	1 pM~150 nM	48 fM	5
Surface enhanced Raman spectroscopy	DNA-silver metallization	0.1 nM~1 μM	34 pM	6
Colorimetry	The target-related dispersion/aggregation of Au nanorods	0~60 nM	1.47 nM	7
Colorimetry	Palindromic molecule beacon-based cascade amplification	10 pM~200 nM	10 pM	8
Electrochemiluminescence	Au NPs@PDA@CuInZnS QDs nanocomposites	0.1~15 nM	0.03 nM	9
Electrochemiluminescence	Based on DNA walkers and AuPd nanomaterials	100 aM~1 nM	31.9 aM	10

RGB readout of fluorescence imaging	Based on PS@Au microspheres and duplex specific nuclease	1 pM~1 nM	50 fM	11
Smartphone-integrated colorimetry	Target-switched artificial biochemical circuit and DNAzyme	5~5×10^4 fM	1.2 fM	This work

Table S3. Target DNA determination in human serum samples with this method.

Sample number	Added	Detected by UV method	Recoveries（%, n=6）	Detected by smartphone	Recoveries（%, n=6）
1	100 fM	93.2 fM	93.2	90.4 fM	90.4
2	500 fM	523.3fM	104.7	535.7 fM	107.1
3	3 pM	2.89 pM	96.3	2.83 pM	94.3

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