Supporting Information

Homogeneous fluorescent biosensing strategy for highly sensitive detection of DNA based on programmed entropy-driven strand displacement reaction and DNAzyme for dual recycling amplification

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Table S1. DNA sequences employed in this work (in 5′ to 3′ Direction)

Oligonucleotide [a]	Sequence (5'-3') [b]
F	AGCCGGTCGAATAGCTCAGCCCTTCATCACACTGGA
L	GAGTCTTCCAGTGTGATGAAGGGCTGAGCTATTCGACCG GCTCGGA
H	CCCTTCATCACACTGGA
E	CTAGTCAG*TCCGAGCCGGTCGAA*TAGCTCAG
S	FAM-CTGAGCTArAGCTGACTAGAT-TAM
p53 gene	TCATCACACTGGAAGACTC
SM	TCATCACAATGGAAGACTC
DM	TCATCACACTGGATCACTC
NC	GACGTCAGACTTCCTGCGA

[a] F, Fuel strand; L; Linker strand; H, Helper strand; E, DNAzyme strand; S, Substrate strand; SM, single-base mismatched oligonucleotides; DM, double-base mismatched oligonucleotides; NC, non-complementary oligonucleotides. [b] The italic portion of E represents the catalytic core of DNAzyme; The underline portions represent mutation bases in target p53 gene. The red portions denote the sequences of toeholds on linker strand for target p53 gene and fuel strand, respectively.

Optimization of method

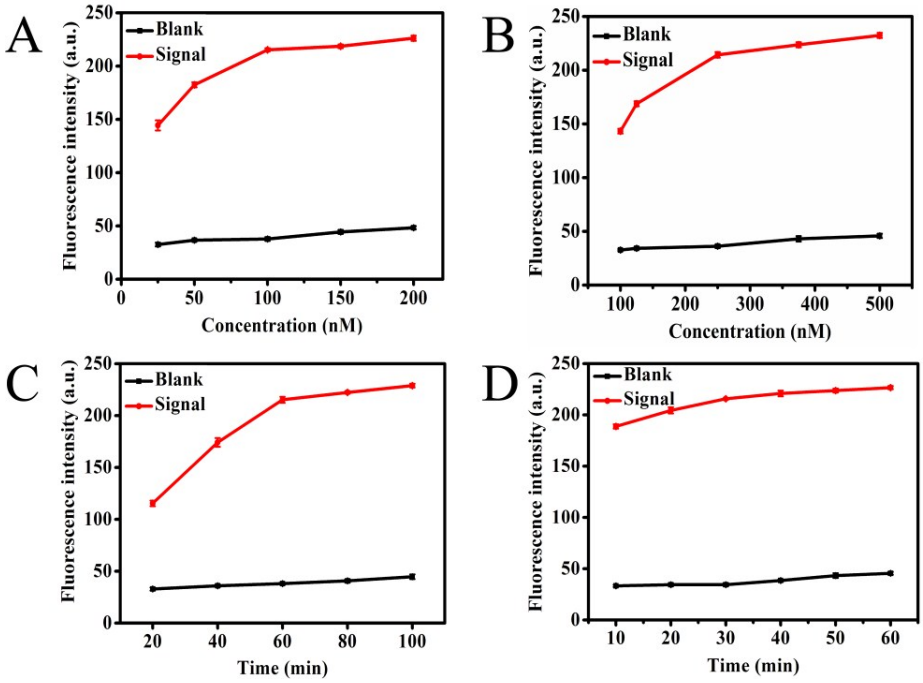

Fig. S1 Optimizations of experimental parameters: (A) concentrations of complex, (B) concentrations of S, (C) ESDRs interaction time, and (D) DNAzyme cleavage time.

To minimize the background signal and achieve the best performance of the sensing system, the crucial experimental conditions including the concentration of the three-stranded complex and DNAzyme substrate, the time of ESDR and the DNAzyme cleavage were investigated. 10 nM target DNA was examined by the ESDR and DNAzyme amplification system. Since the ESDR is a dynamic equilibrium process, it could be strongly influenced by the concentration of three-stranded complex. As shown in Fig. S1A, the fluorescence intensity of the sensing system increased with the increasing concentration of the three-stranded complex, and tended to achieve a stationary phase at 100 nM. With the further increase of concentration of the three-stranded complex, the background signal increased accordingly, indicating that the higher concentration of three-stranded complex increased the target DNA catalyzed reaction and the nonspecific reaction. Therefore, the concentration of the three-stranded complex was fixed at 100 nM for the following experiments. Similar to the shear action of proteases, the splicing process of DNAzyme would achieve stationary phase with the increasing concentration of substrate. As expected, the fluorescence signal achieved stationary phase at 250 nM. Therefore, the optimal concentration of DNAzyme was chose at 250 nM (Fig. S1B). In the following, the ESDR reaction time was inspected. The fluorescence intensity of the sensing system increased with the extension of ESDR reaction time, and arrived at the platform at 60 min (Fig. S1C), indicating that this nanoassembly reaction reached an equilibrium. As a consequence, the optimized ESDR reaction time was chosen as 60 min. The fluorescence signal also increased with the increment of DNAzyme cleavage time, and nearly reached the maximum at 30 min, but the background signal increased with the increase of cleavage time (Fig. S1D). To attain the optimum signal-to-noise ratio, the time for DNAzyme cleavage was optimized as 30 min.