Supporting Information

Cascade Signal Amplification-Based Fluorescent Biosensor Utilizing Exonuclease III and Self-Locking DNAzyme Synergy for microRNA Detection

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Table S1 Oligonucleotide sequences *All sequences are listed 5′→3′. Modifications: FAM = carboxyfluorescein; BHQ1 = Black Hole Quencher 1; rA = riboadenosine; rU = ribouridine.*

Name	Sequence(5'-3')
HP	AAAAA GCTAA AAAAA AAAAA AAACC CCCCC CTTTT TTTTT TTTTT TAGCT TTTTA ACCCC TATCA CGATT AGCAT T
SLD	CACTT CTTTT TTTTT TTTTT TTAGC TTTTT TTTCC TTGGG GAAAG GCTAG CTACA ACGAA GAAGT G
DNAzyme1	CACTT CTACC CCTAT CACGA TTAGC ATTAA TTTCC TTGGG GAAAG GCTAG CTACA ACGAA GAAGT G
Input	AAAAA GCTAA AAAAA AAAAA AAACC CCCCC C
Input1	UUAAU GCUAA UCGUG AUAGG GU
FQ reporter	FAM-CACTT CTrArUT TCCCC-BHQ1
miRNA-155	UUAAU GCUAA UCGUG AUAGG GGUU
miRNA-21	UAGCU UAUCA GACUG AUGUU GA
miRNA-15b	UAGCA GCACA UCAUG GUUUA CA

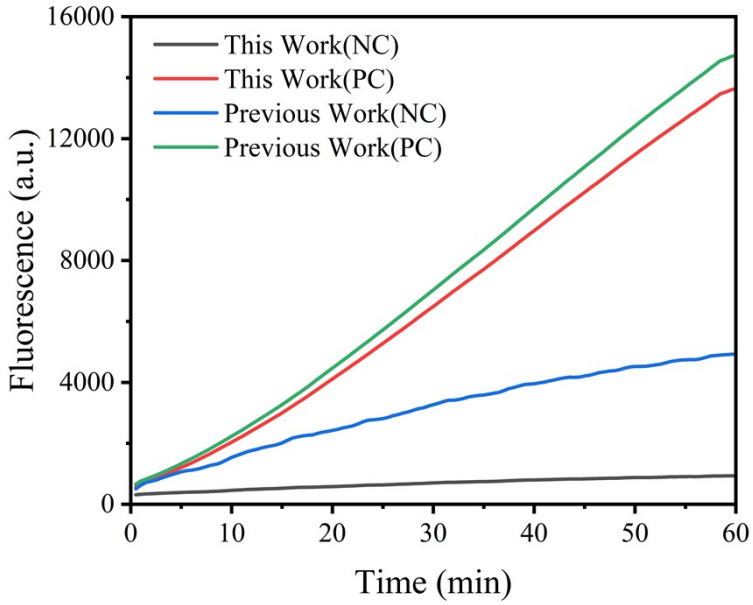

Fig. S1 Comparison of the SLD structure designed in this study versus the previous work. The introduction of additional complementary bases within the locking domain of our SLD effectively reduces signal leakage. Black line: NC of the DNAzyme structure in this work. Red line: PC of the DNAzyme structure in this work. Blue line: NC of the DNAzyme (DNAzyme1, activated by Input1) structure from the previous work. Green line: PC of the DNAzyme structure from the previous work. A significant improvement is observed, with the F1/F0 ratio increasing from 4:1 to over 20:1. The detailed nucleotide sequences are provided in Table S1. (PC: Positive Control; NC: Negative Control)

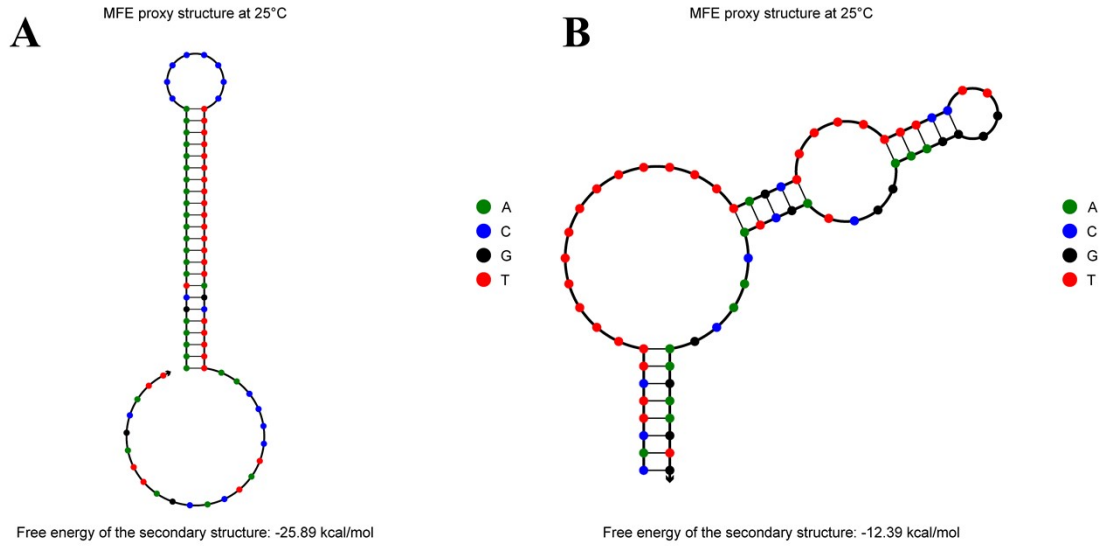

Fig. S2 Simulated secondary structures of DNA motifs at 25°C. (A) Predicted secondary structure of HP (Hairpin DNA). (B) Predicted secondary structure of SLD (Self-locked DNAzyme). *Structures were energy-minimized using NUPACK under physiological buffer conditions (25°C).*

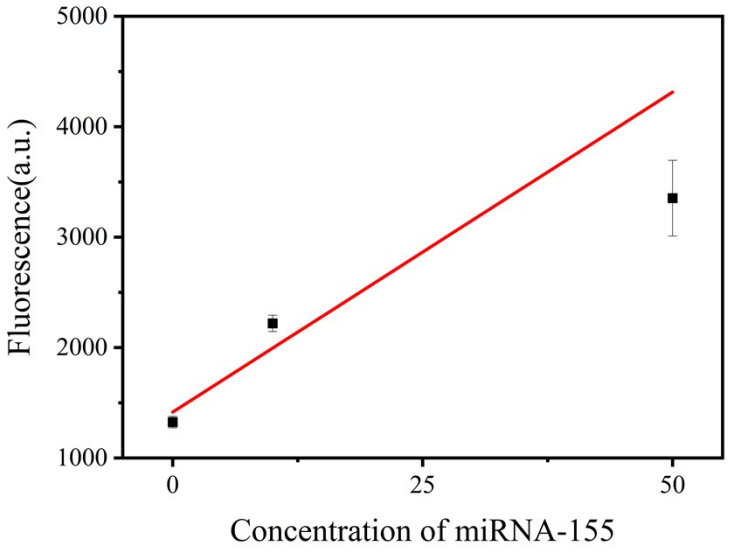

Fig. S3 Linear calibration curve ($y = 57.963X + 1415.077$, $R^2 = 0.836$). The calibration curve was plotted for the range 0 to 50 fM.