Supporting Information

Engineering high-performance hairpin stacking circuits for logic gate operation and highly sensitive biosensing assay of microRNA

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15 **Table S1.** Oligonucleotides used in the present work

Oligonucleotides [a]	Sequence (5'-3')[b]
miR-21	UAGCU-UAUCA-GACU-GAUGUUGA
miR-155	UUAAU-GCUAA-UCGU-GAUAGGGGU
Catalyst	TAGCT-TATCA-GACT-GATGTTGA
H1	TCAACATC-AGTC-TGATA-AGCTAGC-TCCAC-ACAAC-GCTAGCT-TATCA-GACT
H1-T7	TCAACAT-CAGTC-TGATA-AGCTAGC-TCCAC-ACAAC-GCTAGCT-TATCA-GACTG
H1-T6	TCAACA-TCAGTC-TGATA-AGCTAGC-TCCAC-ACAAC-GCTAGCT-TATCA-GACTGA
H1-T5	TCAAC-ATCAGTC-TGATA-AGCTAGC-TCCAC-ACAAC-GCTAGCT-TATCA-GACTGAT
H1S2	TCAACATC-AGTC-TGATA-AGCTAGC-TCCAG-TCAAC-GCTAGCT-TATCA-GACT
H2	TGATA-AGCTAGC-GTTGT-GTGGA-GACT-GATGTTGA-TCCAC-ACAAC-GCTAGCT-CAC
H2S1	TGATA-AGCTAGC-GTTGT-GTGGA-GACT-GATCTTGA-TCCAC-ACAAC-GCTAGCT-CAC
H2S2	TGATA-AGCTAGC-GTTGT-GTGGA-GACT-GATCATGA-TCCAC-ACAAC-GCTAGCT-CAC
H2S3	TGATA-AGCTAGC-GTTGT-GTGGA-GACT-GAACATGA-TCCAC-ACAAC-GCTAGCT-CAC
H2D2	TGATA-AGCTAGC-GTTGT-GTGGA-GACT-GAT_ _TGA-TCCAC-ACAAC-GCTAGCT-CAC
H2I2	TGATA-AGCTAGC-GTTGT-GTGGA-GACT-GATGAATTGA-TCCAC-ACAAC-GCTAGCT-CAC
H3	GTTGTG-TGGA-TCAACATC-AGTC-GCTAGCT-TATCA-GACT-GATGTTGA-TCCA
H3-T5	GTTGT-GTGGA-TCAACATC-AGTC-GCTAGCT-TATCA-GACT-GATGTTGA-TCCAC
H3-T7	GTTGTGT-GGA-TCAACATC-AGTC-GCTAGCT-TATCA-GACT-GATGTTGA-TCC
H3-T8	GTTGTGTG-GA-TCAACATC-AGTC-GCTAGCT-TATCA-GACT-GATGTTGA-TC
H3S2	GTTGTG-TGGA-TCAACATC-AGTC-GCTAGCT-ATTCA-GACT-GATGTTGA-TCCA
Capture probe	SH-(CH2)6-TTTTT-ACGTGTG-AGCTAGC
L1	TCAACATC-AGTC-TGATA-AGCTA
L2	TAGCT-TATCA-GACT-GATGTTGA

L3	TAGCT-TATCAGA- CGGTCGGTGAGAGTGG
L4	CCACTCTCACCGACCG-CTGATGTTGA
SM miRNA	UAGCUUAUCAGACUGAUGUUUA
DM miRNA	UAGCUUAUCAGACUGAUUUUUA
NC miRNA	AUUGAAUAUUCUUAUUAUAAUU

[a] T, Toehold; S, substitution; I, insertion; D, deletion; SM, single-base-mismatched miRNA; DM, double-base mismatch miRNA; NC, non-complementary miRNA. eg, T5 represents the five bases of toehold; S2 represents the two bases were substituted in loop domain of hairpins.

[b] The underline portion represents the mismatched bases in target miRNA. The substitution, insertion, and deletion bases of hairpins in red.

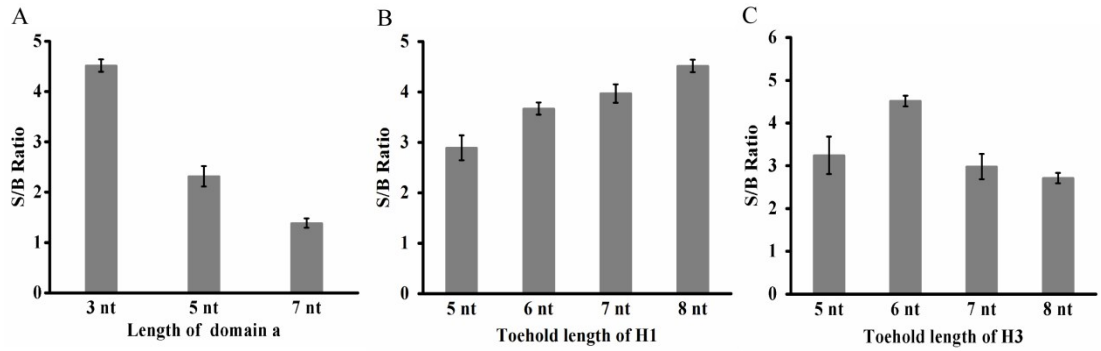

Fig. S1. The effect of (A) the length of domain a, (B) the toehold of H1, (C) the toehold of H3 on the signal-to-background (S/B) ratio. Error bars are standard deviation obtained from three independent experiments. The flow rate of the SPR experiment was set to 1 μL min^{-1}. The freshly prepared reaction solution was injected into the flow cell and flowed for 30 min.

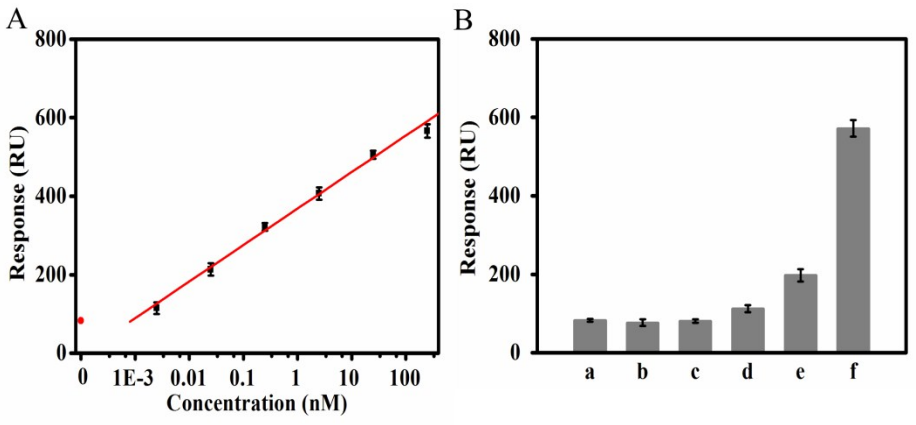

Fig. S2. (A) The calibration curve of SPR response signals versus target at 0.0025, 0.025, 0.25, 2.5, 25, and 250 nM. (B) SPR response signal respectively corresponding to various oligonucleotides (a) blank, (b) miR-155, (c) NC miRNA-21, (d) DM miRNA-21, (e) and SM miRNA-21, (f) miRNA-21. Error bars are standard deviation obtained from three independent experiments.

Specificity, reproducibility and reusability of the strategy

To test the specificity of the developed method, we used five different oligonucleotides, including matched, single-base mismatched (SM), double-base mismatch (DM), non-complementary (NC) miRNA-21 and miRNA-155 sequences at the same concentration of 0.25 μM. As shown in Figure S2B, the presence of miRNA-155, DM, and NC miRNA-21 showed negligible response signals compared with that of the blank test (background signal with the absence of the target miRNA-21), respectively. However, the presence of the target miRNA-21 resulted in a significantly enhanced response signal compared with that of the SM miRNA-21. These results indicated that the biosensor displayed excellent specificity for the determination of target miRNA-21. In addition, the repeatability of the developed enzyme-free and label-free SPR strategy was studied through five replicate measurements for miRNA-21 at 0.1 nM and 100 nM. The relative standard deviations (RSDs) were less than 5%, suggesting this method had good repeatability. Effective regeneration is important to eliminate carry-over of analyte between analysis cycles and reuse SPR biosensor. To further estimate the reusability of the developed SPR method, repeated regenerations were carried out on the same sensor chip. The sensor chip could be regenerated 100 times while the SPR response signal only decreased less than 10%, suggesting the same sensor chip could be reused 100 times at least (depending on the error). Therefore, the sensor chip can be reused by removing all bound analyte.

Table S2. Comparison of different nucleic acid detection methods on SPR biosensing platform

Platform	Amplification strategy [a]	Dynamic range	LOD	Reference
SPR	Super-sandwich and streptavidin	10 pM-1 μM	9 pM	1
SPR	PER	100 pM-250 nM	100 pM	2
SPR	HCR	0.5 fM-500 fM	0.3 fM	3
SPR	Streptavidin functionalized AuNRs	0.1 pM-100 pM	45 fM	4
SPR	Hairpin stacking circuits	2.5 pM-250 nM	1.5 pM	This work

[a] PER, polymerization extension reaction; HCR, hybridization chain reaction; AuNRs, gold nanorods.

Table S3. Recoveries of the developed SPR platform via spiking targets in salmon sperm DNA samples

samples	spiked (nM)	measured (nM)	Recovery (%)	RSD (%)
1	0.01	0.011	110	5.5
2	0.1	0.098	98	2.1
3	10	10.3	103	3.6
4	100	108.6	108.6	4.7

Reference:

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