Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

A highly sensitive SPRi biosensing strategy for simultaneous detection of multiplex miRNAs based on strand displacement amplification and AuNPs signal enhancement

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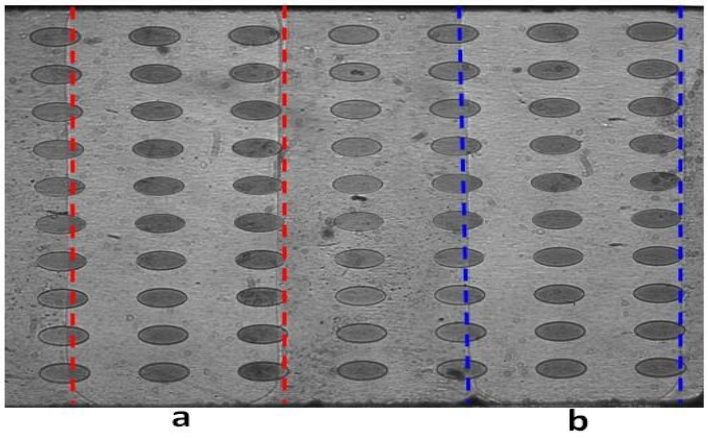

Fig. S1. Image of dual channel SPRi.

The dual channel SPRi biosensor for detection of multiplex miRNAs. The channel 1 was for detection of miR-21 (Fig. S1. a), and the channel 2 was for detection of miR-192 (Fig. S1. b).

The TEM image of the prepared AuNPs is shown in Fig. S2A. It displayed spherical and homogeneous distribution with a mean size of 14 nm in diameter. The AuNPs and DNA-functionalized AuNPs were characterized using UV-vis absorption spectra (Fig. S2B). With the addition of 0.5 M NaCl, the color of the AuNPs solution changed from deep red to violet (Fig. S2B (c)), indicating the NaCl-induced aggregation of AuNPs. However, the color of the DNA-functionalized AuNPs remained deep red with the addition of 0.5 M NaCl (Fig. S2B (a)), owing to the repulsion among the DNA-functionalized AuNPs enhanced the stability of the AuNPs against NaCl-induced aggregation. These results indicated that the labeling of AuNPs with thiol-modified oligonucleotides was successfully achieved.

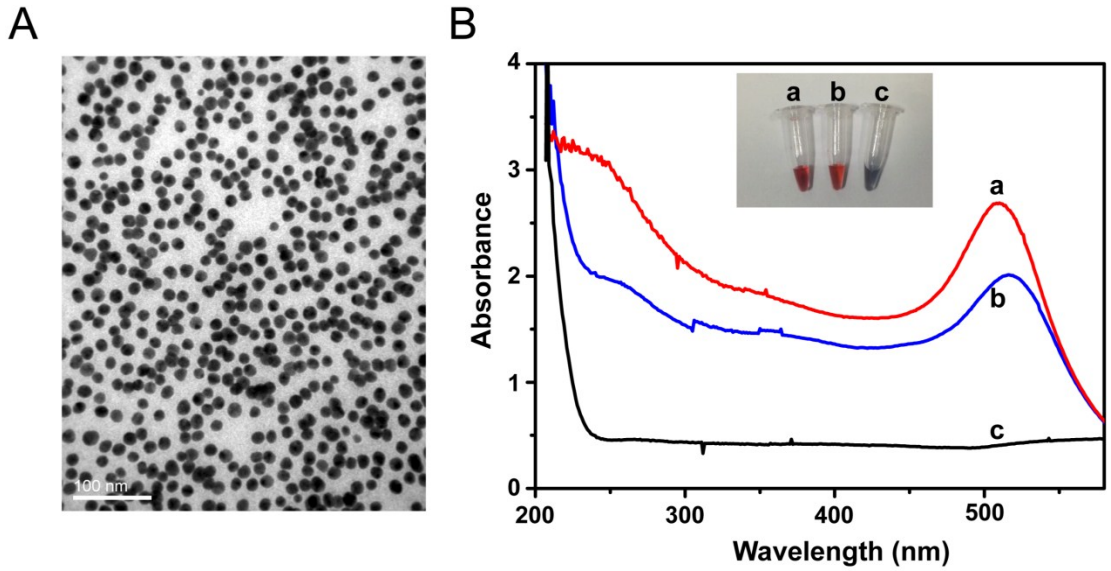

Fig. S2. (A) TEM image of AuNPs. Scale bars = 100 nm, (B) UV–vis absorption spectra of DNA-functionalized AuNPs after addition of 0.5 M NaCl (a), AuNPs (b), and AuNPs + 0.5 M NaCl (c).

Table S1. DNA and miRNA sequences employed in this work.

Oligonucleotides	Sequences (5'-3')
miR-192	CUGACCUAUGAAUUGACAGCC
miR-21	UAGCUUAUCAGACUGAUGUUGA
Single-base mismatch miR-192[a]	CUGACCUAUGAAUUGACAUCC
Double-base mismatch miR-192[a]	CUGACCUAUGAAUUUACAUCC
Single-base mismatch miR-21[a]	UAGCUUAUCAGACUGAUGUUUA
Double-base mismatch miR-21[a]	UAGCUUAUCAGACUGAUUUUUA
H1[b]	GTCAGATGAATTCGTGTGAGAGCACCTCAGCCGCG CG *TCAACATCAGTCTGATAAGCTA*GACGCGCG-$(CH_2)_6$
H2[b]	GTCAGATGAATTCGTGTGAGAGCACCTCAGCCGCG CG *GGCTGTCAATTCATAGGTCAG*GACGCGCG-$(CH_2)_6$
miR-222	AGCUACAUCUGGCUACUGGGUCUC
Non-complementary sequence	AUUGAAUAUUCUUAUUAUAAUU
Capture probe	TGTGAGAGCACCTCATTTTTTTT-SH
Detection probe	SH-$(CH_2)_6$- TTTTTTGTCAGATGAATTCG

[a]The underline portion represents mutation bases in target miRNA. [b]The italic portion of hairpin represents complementary sequences to miR-21 and miR-192 respectively.

Table S2. Comparison of the developed method with others in the detection of miRNA.

SPR platform	Amplification strategy	LOD
SPRi	Orthogonal signal amplification[1]	0.5 fM
SPRi	AuNPs and streptavidin amplification[2]	0.5 pM
Biacore X	Streptavidin and supersandwich amplification[3]	9.0 pM
Biacore X	Streptavidin and CHA[a] amplification[4]	1.0 pM
SPRi	Silica nanoparticles amplification[5]	0.1 pM
SPRi	Poly(A) polymerase and AuNPs[6]	10 fM
Home-built SPRi	AuNPs and SDA[b] amplification	0.15 pM

[a]Catalytic hairpin assembly; [b]Strand displacement amplification.

Table S3. Comparison of the developed method with the scanometric array in washing steps.

	Washing times	Solutions	Immersion times	Solutions
Scanometric array[7]	3	1. pre-warmed (52°C) 2 × SSC 2. pre-warmed (56°C) 2 × SSC 3. nanopure water	2	2 × SSC, phosphate buffered saline, and nanopure water
SPRi	2	Running buffer	0	

The developed SPRi biosensor showed simple washing steps compared with the scanometric array. As shown in Table S3, the scanometric array needed three washing times with different solutions, while SPRi needed two washing times with the same solution. In addition, the scanometric array also needed two immersion times with three different solutions, while SPRi did not need.

Table S4. The recoveries via spiking miR-21 and miR-192 into 10% diluted fetal bovine serum respectively.

Sample	Spiking value (pM)	Assayed value (pM)	Recovery (%)
Spike-miR-21(1)	1	1.10	110.0
Spike-miR-21(2)	10	10.6	106.0
Spike-miR-21(3)	100	104.4	104.4
Spike-miR-192 (1)	1	1.08	108.0
Spike-miR-192 (2)	10	10.7	107.0
Spike-miR-192 (3)	100	103.3	103.3

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