An innovative “unlocked mechanism” by a double key avenue for one-pot detection of microRNA-21 and microRNA-141

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Table S1. Oligonucleotide sequences of miRNAs (miR-21, miR-141, miR-200b, Let-7d, negative control miRNA (NC), single mismatched miRNA (SM)) and the modified DNA probes (HD-21, HD-141, ssDNA-21, ssDNA-141, FAM-ssDNA, Cy5-ssDNA) for miRNA detection.

Name	Sequence (5'-3')
HD-21	CCTCAACATCAGTCTGATAAGCTAGTTGAGGTTTTTTTTTT-NH_2
HD-141	ATTGTGACAGACCATTTCTACCACAATTTTTTTTTTTT-NH_2
ssDNA-21	AAAACCTCAACC-NH_2
ssDNA-141	AAAAATTGTTTT-NH_2
FAM-ssDNA	FAM-AAAACCTCAACC-NH_2
Cy5-ssDNA	AAAACCTCAACC-Cy5
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-141	UAACACUGUCUGGUAAAGAUGG
NC	UUGUACUACACAAAAGUACUG
miR-200b	UAAUACUGCCUGGUAAUGAUGA
Let-7d	AGAGGUAGUAGGUUGCAUAGUU
SM	UAGCUUAUCGGACUGAUGUUGA

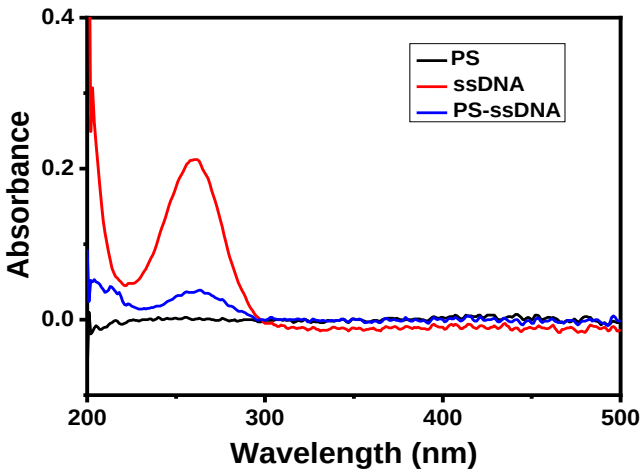


Figure S1. The UV-vis absorption spectra of PS (black line), ssDNA (red line) and the purified PS-ssDNA probes (blue line).

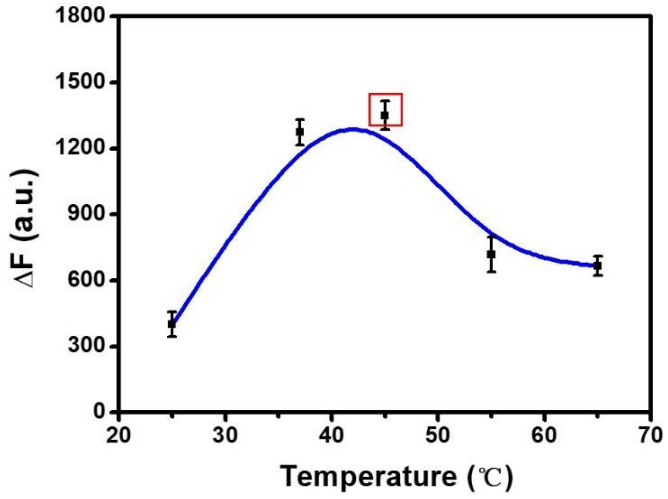
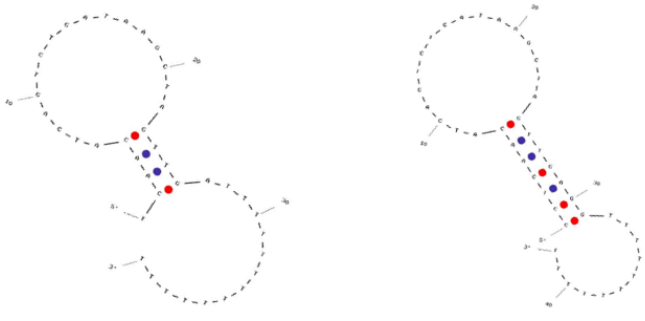


Figure S2. Optimization test of the reaction temperature for the double key unlocking detection assay.

Table S2. The hairpin DNA probes of HD-21(a) and HD-21(b)

Name	Sequence (5'-3')
HD-21 (a)	CCTCAACATCAGTCTGATAAGCTAGTTGAGGTTTTTTTTTTT-NH_2
HD-21 (b)	ATTGTGACAGACCATTTCTACCACAATTTTTTTTTTTTT-NH_2



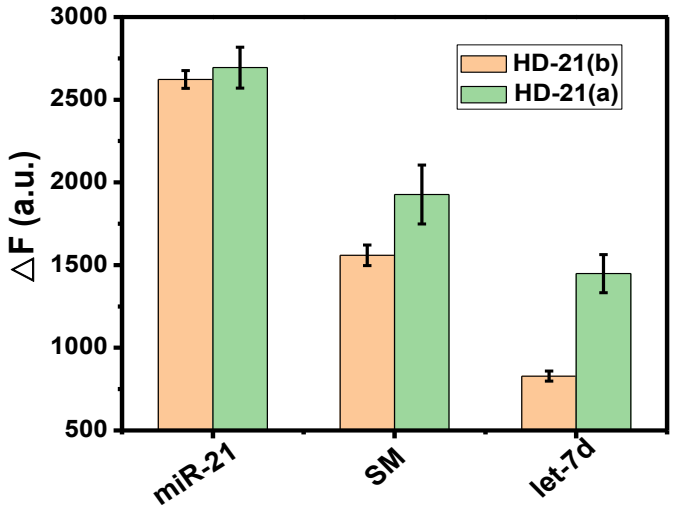


Figure S3. The specificity test of different hairpin DNA probes for miR-21detection.

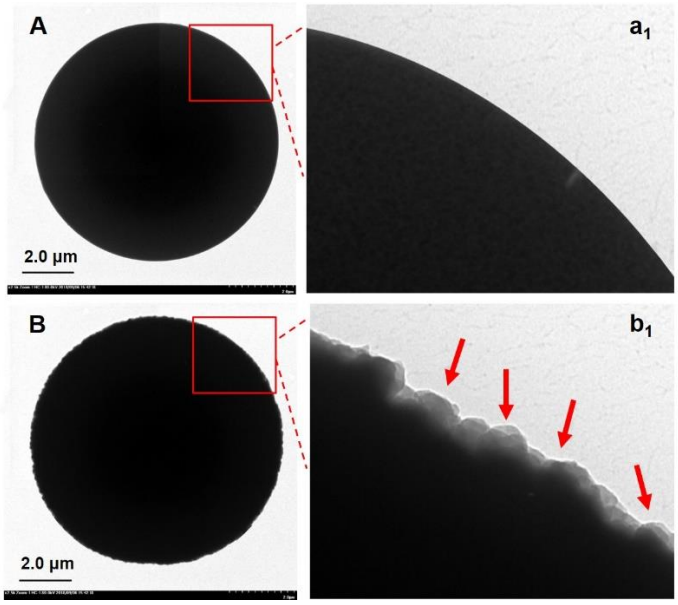


Figure S4. TEM images of PS microparticles before modification **(A, a_1)** and PS-ssDNA-FS conjugates after modification **(B, b_1)**. In comparison with PS microparticles of smooth surface, we observe rough surface of PS-ssDNA-FS conjugates after reactions. The small beads (indicated by red arrows) on the surface of PS microparticles (10 μm) are FS particles (50 nm).

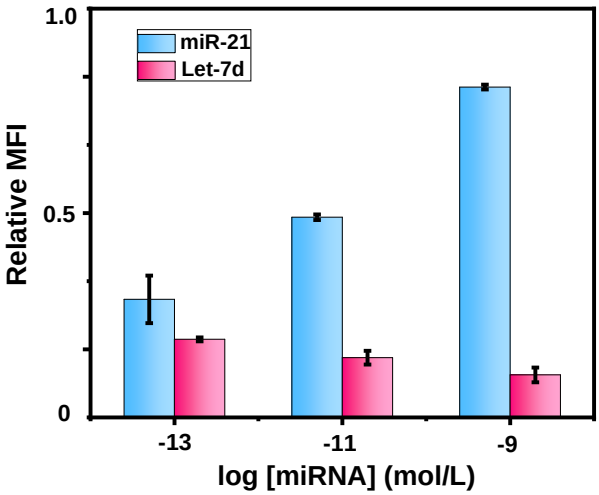


Figure S5. Feasibility study of our assay. Different concentrations of miR-21 and Let-7d were used for the flow cytometry assay. With the increase of miRNA content, the fluorescence intensity detected for miR-21 was gradually increased, but the fluorescence intensity for Let-7d remained basically unchanged.

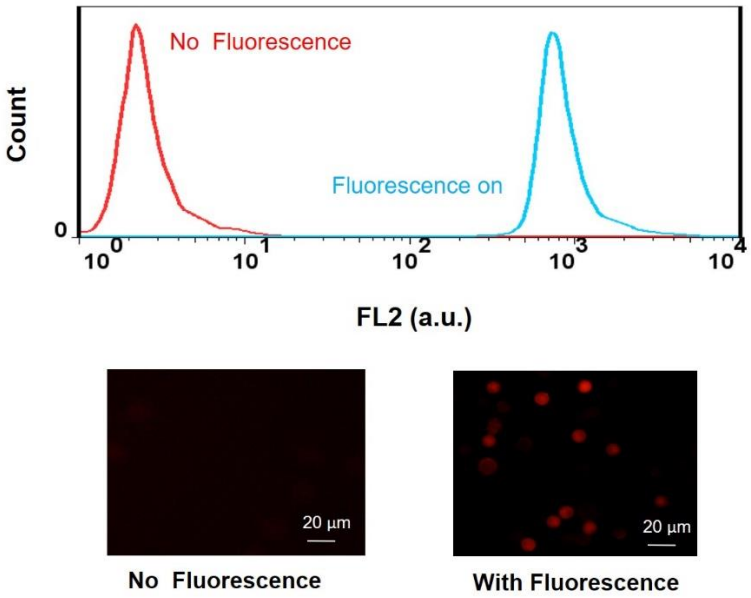


Figure S6. Detections for miR-141. Flow cytometry detections and fluorescent images of probe 2 for miR-141 detection before (no fluorescence) and after (with fluorescence) reactions.

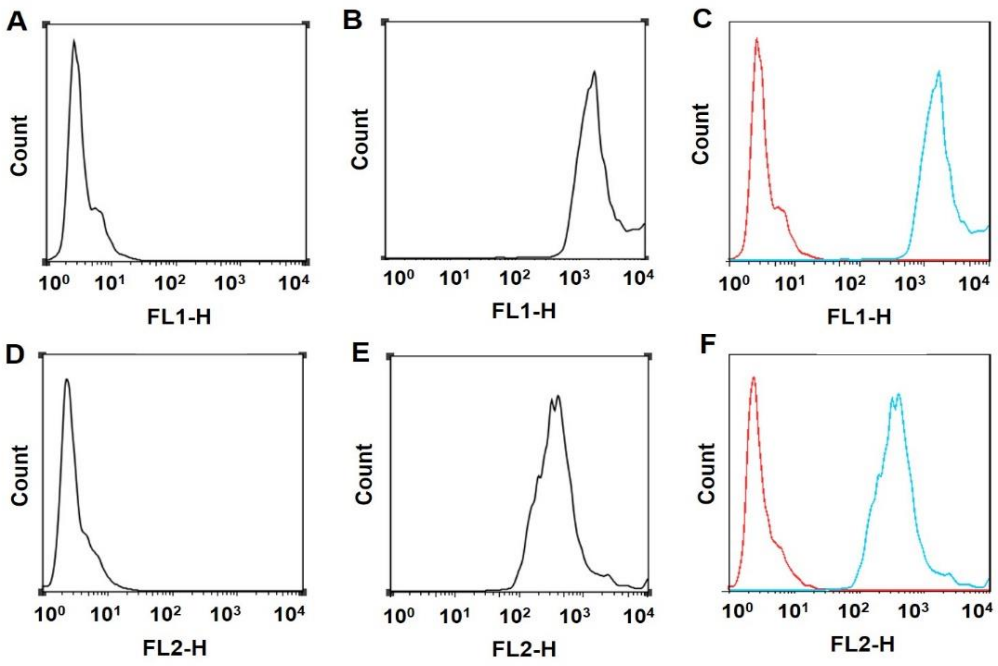


Figure S7. Histograms of fluorescence response of the multiplexed analysis of miR-21(FL1-H) and miR-141 (FL2-H) assay. Figure S7C and **S7F** were respectively merged by the results of **A/B and D/E**.

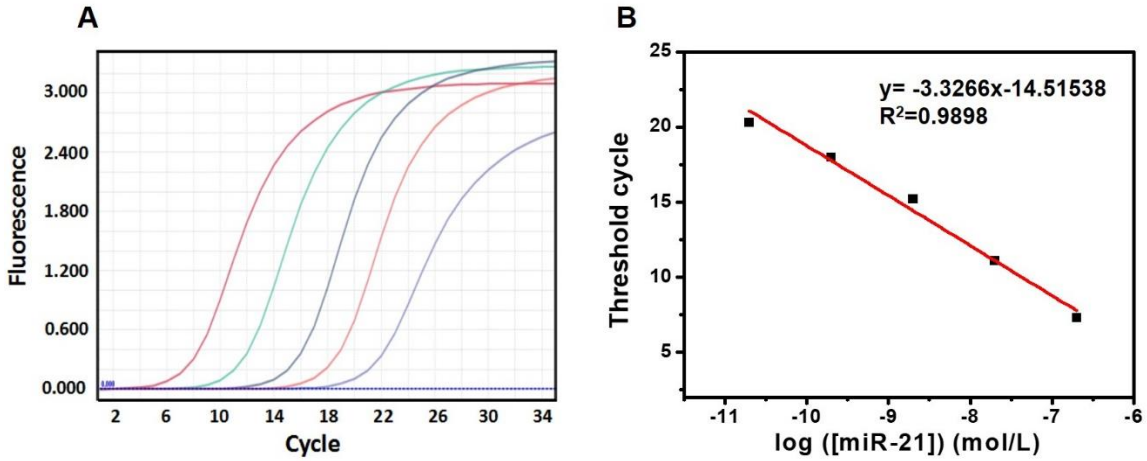


Figure S8. Real simple detection by qRT-PCR. (A) The real-time qRT-PCR curves of different concentrations of target detection. **(B)** The linear relationship of the miRNA concentrations and the threshold cycle.

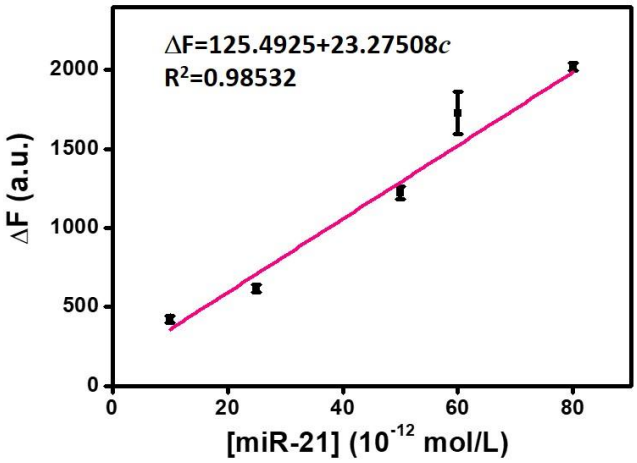


Figure S9. Calibration curve of ΔF value vs. concentration of miRNA-21 using the proposed method serviced for the miRNA-21 detection in human serum samples. A linear relationship between ΔF and different concentrations of miR-21 (10, 25, 50, 60, 80 pM) is observed, and the linear equation is ΔF = 125.4925 + 23.27508*c* (R^2 = 0.98532).

Table S3. Analytical recoveries of the proposed assay in detecting miR-21 spiked human serum samples.

miRNA-21			
Sample	Founded (10^{-12} M)	Recovery (%)	RSD (%)
1	48.35667	96.71	6.25%
2	44.64333	89.29	10.67%
3	48.90	97.8	7.68%
4	54.32667	108.65	4.04%
5	47.52667	95.05	11.75%

Table S4. The detection limit for miRNA of this proposed method and other detection strategies

Method	Materials	LOD	Multiplex Detection	Reference
Fluorescence	Microbeads and DSN	3.39 fM	Yes	Our work
Fluorescence	Molecular Beacons and DSN	0.4 pM	No	[1]
Colorimetry	Cationic Polythiophene Derivative	10 nM	No	[2]
Chemiluminescent	Magnetic Beads and DSN	10 fM	No	[3]
MRS	magnetic Beads and DSN	5 fM	No	[4]
Electrochemistry	Graphene	60 fM	No	[5]
Electrochemistry	DNA CPs and DSN	1 fM	No	[6]

References

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