Supporting information

Assensing system constructed by combining structure switchable molecular beacon with nicking-enhanced rolling circle amplification for highly sensitive miRNA detection

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Table S1. Oligonucleotide sequences designed in this study.

Note	Sequence (5'→3')
Structure switchable molecular beacon (SMB)	AAAGGGAGGCTTGACCGGACCTCTGGGACT(iDabcyld)GT CACGCAGAGGTCCGAGTGACGTGACA(FAM)
Padlock probe (PP)	CTGATAAGCTAGCTGAGGGTCCCAGAGGTCCGGTCAAG CCTCCCTTTTTTGTCCCAGAGGTCCGGTCAAGCCTCCCTT TTTTGTCCCAGAGGTCCGGTCAAGCCTCCCTTTGCTGAG GTCAACATCAGT
Ligation template 1 (LT1)	GCTTATCAGACTGATGTT
Ligation template 2 (LT2)	AGCTTATCAGACTGATGTTG
Ligation template 3 (LT3)	TAGCTTATCAGACTGATGTTGA
miRNA-21	UAGCUUAUCAGACTGAUGUUGA
miRNA-21D	TAGCTTATCAGACTGATGTTGA
miRNA-141D	CATCTTCCAGTACAGTGTTGG
miRNA-31D	AGGCAAGATGCTGGCATAGCT
miRNA-122D	TGGAGTGTGACAATGGTGTTTG
miRNA-26aD	TTCAAGTAATCCAGGATAGGCT

The repetitive fragments in red in PP strand are designed to hybridize with the red fragment of SMB. The blue fragments in SMB are complementary to each other when the red fragment is hybridized to cyclized PP. For padlock probe (PP), the fragments with grey background are the recognization sites of Nt.BbvcI, while the fragments with yellow backgrond are binding site of miRNA-21 after cyclization, and the underlined fragments are capable of hybridizing with LP 1. Because DNA oligonucleotides are more stable than corresponding RNA strands, the synthesized DNA counterparts are used during *in vitro* tests.

Table S2. Comparison of assay ability of SMB assembly and literature sensing systems.

	Method	Target	LOD	References
1	Localized DNA cascade reaction (LDCR)	miRNA-21	85.3 pM	[1]
2	Nicking-enhanced rolling circle amplification (N-RCA)	Let-7a	10 pM	[2]
3	Label-free multifunctional MB (LMMB)	P53 gene	20 nM	[3]
4	Aptamer-tethered, DNAzyme-embedded MB (ADB)	miRNA-21	2 nM	[4]
5	Gold nanoparticle-based molecular beacon (Au NP-MB)	P16, P21, P53 genes	0.1 nM	[5]
6	Common molecular beacon	miRNA-21	0.5 nM	[6]
7	Duplex-specific nuclease-assisted CRISPR-Cas12a strategy	miRNA-21 miRNA-205	2.4 pM 1.1 pM	[7]
8	Backbone-modified molecular beacons	let-7a	0.4 pM	[8]
9	Flexible molecular beacon-combined and Nickase-enhanced rolling circle amplification (SMB-NRCA)	miRNA-21	1 pM	This work

Table S3. Recovery studies of miRNA-21

Sample	Added (nM)	Measured (nM)	Recovery	RSD
D1	5.00	5.44	108.71%	2.79%
D2	2.00	2.11	105.39%	4.71%
D3	1.00	0.91	91.44%	1.27%
D4	0.50	0.47	93.97%	0.94%
R1	5.00	4.95	98.93%	6.99%
R2	2.00	2.06	103.10%	7.31%
R3	1.00	0.92	91.87%	5.70%
R4	0.50	0.53	106.25%	5.54%

D1-D4 represent the samples prepared with DNA mimic of miR-21, while R1-R4 represent the samples prepared with RNA-based miR-21 with nucleotide base sequence.

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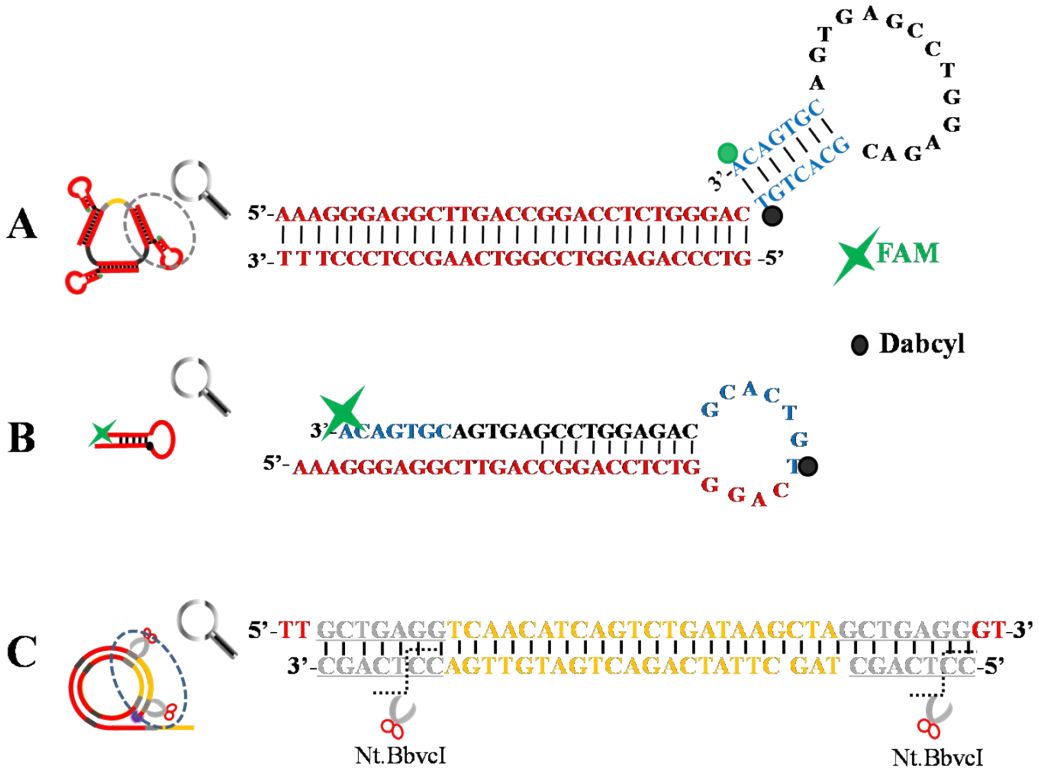
SB. Supporting scheme



Scheme S1. The conformational transition of SMB and recognition site for Nt.BbvcI. (A) When hybridizing to cyclized padlock probe (CPP), SMB can fold into a small hairpin structure, and the FAM fluorescence is quenched by Dabcyl. (B) When being displaced from CPP, the quencher and FAM are separated from each other due to the switching of molecular configuration, restoring the pre-quenched fluorescence. (C) The double-stranded fragments of RCA product/CPP complex contain the recognition sites of Nt.BbvcI and thus can be nicked, producing the new nicks and polymerization primers. As a result, during the next RCA reaction, the midstream product (SNF) and downstream nicked product (LNF) can be displaced by the extended upstream primer.

SC. Supporting figures

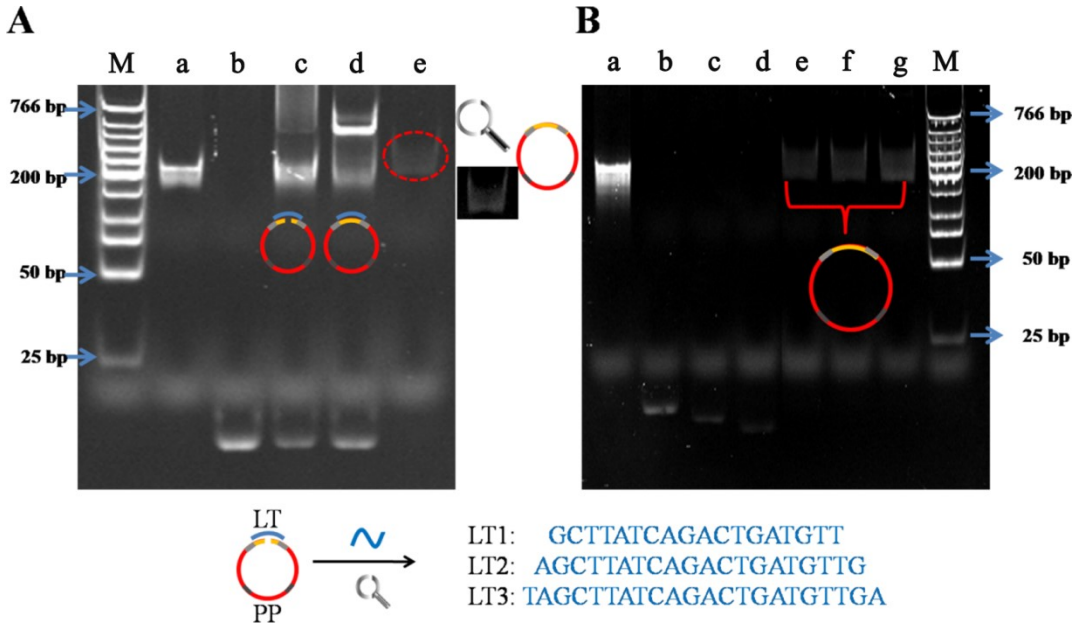


Figure S1. Verification of CPP by 12% native PAGE. (A) The formation of CPP. (a) p-PP (phosphorylated padlock probe); (b) LT1; (c) p-PP + LT1; (d) p-PP + LT1 + T4 DNA ligase; (e) the same as (d) but treated with exo I and exo III. **(B)** Optimization of ligation template. (a) p-PP; (b) LT3; (c) LT2; (d) LT1. The lanes e, f and g were the CPP products corresponding to b, c and d, respectively. The letter "M" means the low weight DNA ladders. The concentrations of p-PP and LT (including LT1, LT2 and LT3) were 500 nM.

Experimental procedure:

To cyclize the p-PP, 1 μL of p-PP (10 μM), 1 μL of LT1 (10 μM), 2 μL of 10×T4 DNA buffer and 16 μL of ddH_2O were mixed together. After annealing at 90 °C for 5 min and cooling to room temperature, 1 μL of T4 DNA ligase (5 U/μL) was added and incubated at 16 °C for 2.5 h, followed by heating at 65 °C for 10 min to inactivate the enzyme. Next, 0.2 μL of exo I (20 U/μL), 0.2 μL of exo III (100 U/μL), 2.7 μL of 10×exo I buffer and 2.7 μL of 10×exo III buffer were added and incubated at 37 °C for 1 h. The ligation product is CPP that is immediately verified by 12% nPAGE analysis according to 2.3. Gel electrophoresis analysis.

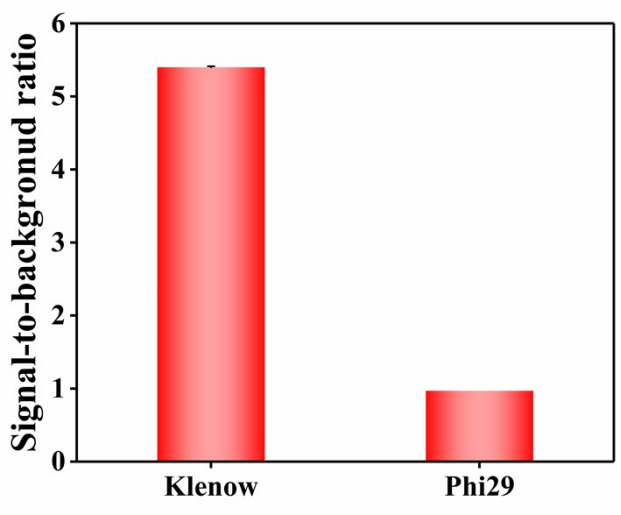


Figure S2. The dependence of assay performance of NCP system on polymerase nature. The S/B ratio was calculated by $F_e/F_b \times 100\%$, in which F_e and $F_b$bstand for the fluorescence intensity at 519 nm in the presence and absence of target miRNA-21, respectively. The concentrations of CPP, SMB, miRNA-21, dNTPs, Klenow DNA polymerase, phi29 polymerase and Nt.BbvcI were 20 nM, 50 nM, 5 nM, 150 nM, 25 U/mL, 25 U/mL and 25 U/mL respectively.

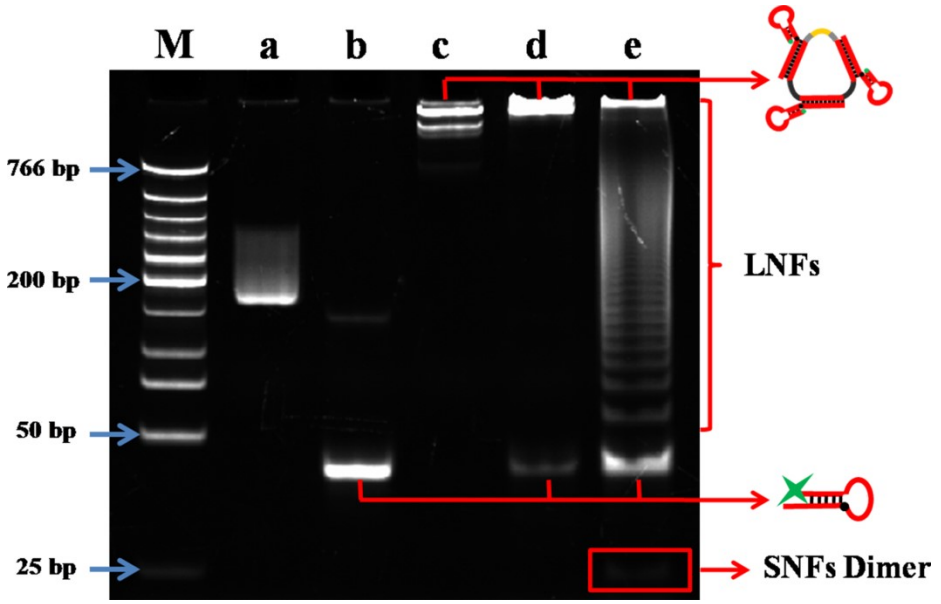


Figure S3. Validation of nicking-based RCA reaction occurring during target detection by nPAGE analysis. NCP system (staining with SYBR Green I). Lane a: CPP; Lane b: SMB; Lane c: CPP + SMB; Lane d: CPP + SMB + target + dNTPs + polymerase; Lane e: CPP + SMBs + target + dNTPs + polymerase + nickase. The reaction system was the same with Figure 1B.

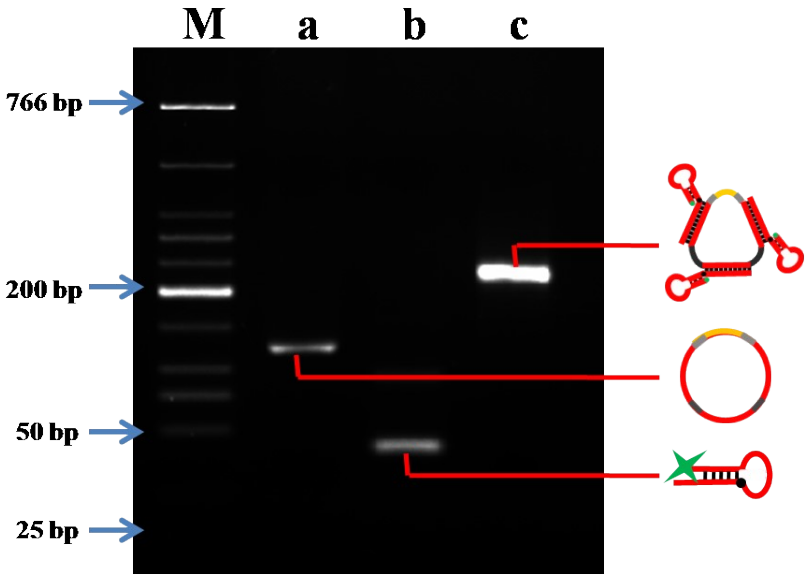


Figure S4. Verification of the formation of NCP probe (CTD/ SMBs complex) by 3% agarose gel electrophoresis. Lane a: CPP; Lane b: SMB; Lane c: CPP + SMBs. The concentrations of CPP and SMB were 200 nM and 500 nM, respectively.

SC. Supporting references

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