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Supporting Information

RNA responsive and catalytic self-assembly of DNA nanostructures for highly sensitive fluorescent detection of microRNA from cancer cells

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Experimental Section

Reagents and Materials: Tris(hydroxymethy-l)aminomethane (Tris), magnesium acetate ($Mg(CH_3COO)_2$), acetic acid (CH_3COOH), chloroform ($CHCl_3$), 2-propanol, ethanol (CH_3CH_2OH), sucrose, xylene cyanol FF, bromophenolblue and eathylenediaminetetraacetic acid (EDTA) were purchased from Kelong Chemicals Inc. (Chengdu, China). The Trizol reagent, synthetic miR-21 and all DNA were obtained from Invitogen Biotechnology Co., Ltd (Shanghai, China).

Table S1 All nucleic acid sequences used in the experiments

Name	Sequence from 5' to 3'
miR-21	UAGCUUAUCAGACUGAUGUUGA
One-base mismatched sequence	UAACUUAUCAGACUGAUGUUGA
Two-base mismatched sequence	UAACUUAUCAGCCUGAUGUUGA
miR-141	UAACACUGUCUGGUAAAGAUGG
miR-199a	ACAGUAGUCUGCACAUUGGUUA

Hairpin probe 1 (H1)	CGTTCAGCACAAGACTGATGTTGAGAATGAGT GAATCAACATCAGTCTGATAAGCTAATGCCT
Hairpin probe 2 (H2)	CGTTCAGCACAAGAATGAGTGAAAGATAGCTT ATCATTCACTCATTCTCAACATCAGATGCCT
Hairpin probe 3 (H3)	CGTTCAGCACAAGATAGCTTATCAGACTGATG TTGATGATAAGCTATCTTTCACTCATATGCCT
T*-t*	AGGCATTCTTGTGCTGAACG-FAM
C	DABCYL-CGTTCAGCACAAGA

Preparation of the dsDNA signal report probes: The dsDNA probes were prepared by mixing the T*-t* stands with the C stands at a molar ratio of 1:1.2 in the TAE/Mg buffer (40 mM Tris, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesicon acetate, pH 8.0). The mixture was heated to 90 °C for 5 min and was allowed to cool down slowly to 25 °C in a period of 2 h to obtain the dsDNA signal probes.

Protocol for sensitive detection of miR-21: Various concentrations of miR-21 were incubated with the mixture of H1 (1 μM), H2 (1 μM), and H3 (1 μM) in TAE/Mg buffer for 4 h at 15 °C. This is followed by the addition of the dsDNA signal probes and further incubation for 1 h. The fluorescence emission spectra of the mixtures were then recorded on a RF-5301PC fluorescence spectrophotometer (Shimadzu, Tokyo, Japan) from 490 to 700 nm at the excitation wavelength of 490 nm. A 150 W Xenon lamp was used as the excitation source and the excitation and emission slit widths were both set at 5 nm.

Non-denatured polyacrylamide gel electrophoresis: The DNA sample solutions were mixed with DNA loading buffer (40% (v/v) sucrose solution, 0.25% (w/v) xylene cyanol FF and 0.25% (w/v) bromophenol blue) on a volume ratio of 5:1 and gel electrophoresis was performed in 1× TBE (pH 8.3) at a 100 V constant voltage for 100 min, followed by

staining with bromide. Gel imagines were taken by a canon digital camera (EOS 550D) under the UV light illumination.

Cell culture and total RNA extraction: All appliances used in the following procedure were soak in 0.1% DEPC solution overnight and dried in an oven after a sterilization process. The solutions used in all experiments were prepared using DEPC treated water. The human breast cancer cell lines (MCF-7) and cervical cancer cell lines (HeLa) were obtained from the cell bank of the type culture collection of the Chinese Academy of Sciences (Shanghai, China) and cultured in RPMI 1640 medium (Thermo Scientific Hyclone) supplemented with 10% fetal bovine serum, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin at 37 °C in a humidified 5% CO_2 incubator. Total RNA samples were extracted from each cell lines by using Trizol Reagent according to the manufacturer's protocol. Briefly, the appropriate amount of Trizol Reagent were added to the cell pellet shaking 3-4 times and then transferred to an RNase-free centrifuge tubes for 5 min at room temperature to ensure complete cell disruption. Phase separation was performed by adding chloroform solution and centrifugation at room temperature. Certain amount of isopropylalcohol solution was then transformed into the above aqueous phase to recover the total RNA after precipitating at room temperature for 10 min. The RNA pellet was washed with 75% ethanol and finally re-dissolved in RNase-free water.

Supplementary Figures:

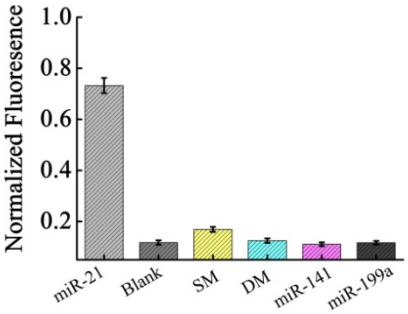

Fig. S1 Selectivity investigation of the proposed detection method for the target miR-21 (10 pM) against other control molecules, one-base mismatched sequence (100 pM), two-base mismatched sequence (100 pM), miR-141 (100 pM) and miR-199a (100 pM)

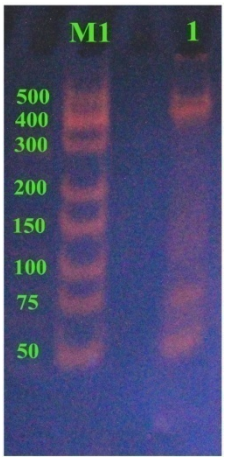

Fig. S2 8 % PAGE characterization of the miR-21 (1 μM)-triggered, catalytic assembly of the hairpin DNAs. Lane 1: the mixture of miR-21, H1 (3 μM), H2 (3 μM) and H3 (3 μM) and the left lane is the marker DNA.

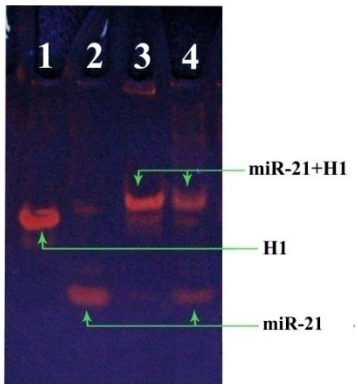

Fig. S3 PAGE (16%) characterization of hybridizations between miR-21 and H1 with different molar ratios. Lane 1 and 2 correspond to miR-21 (1 μM) and H1 (1 μM), respectively. Lane 3: the mixture of miR-21 (1 μM) and H1 (1 μM); Lane 4: the mixture of miR-21 (1 μM) and H1 (0.5 μM).

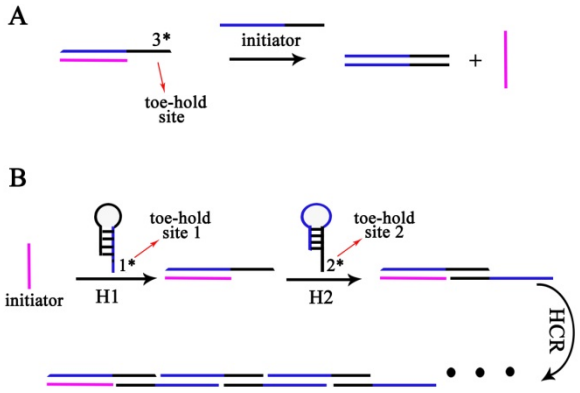

Fig. S4 Schematic representation of (A) Toe-hold strand displacement reaction and (B) Hybridization chain reaction.

Toe-hold strand displacement reaction (TSDR) (Fig. S4A) refers to the extraction of a short, pre-hybridized ssDNA strand from the dsDNA duplex by a fully complementary invading strand upon binding to the single stranded domain (toe-hold, 6-10 nt) of the hybridized dsDNA. HCR (Fig. S4B) refers to series TSDRs between two metastable hairpin DNAs.

Table S2 Comparison of different fluorescent methods for miR-21 detection

Detection Method	Detection Limit (M)	Reference
Quantitative real-time polymerase chain reaction	5.0×10^{-15}	1
Quantitative real-time polymerase chain reaction	1×10^{-15}	2
Enzymatic repairing amplification	1.0×10^{-16}	3
Hairpin-mediated quadratic enzymatic amplification strategy	1×10^{-18} at 4 °C 1×10^{-14} at 37 °C	4
Quantum dots (QDs)-based nanosensors	1×10^{-14}	5
Duplex-specific nuclease signal amplification	3×10^{-13}	6
Rolling-Circle Amplification	1.0×10^{-14}	7
Isothermal Strand Displacement Polymerase Reaction	2.1×10^{-15}	8
DNA nanotechnology	2.5×10^{-17} at 15 °C	This work

Table S3 Recovery tests for miR-21 in human serum samples using the proposed method (n=6).

Sample	Added	Normalized Fluorescence	Found	Rate of recovery (%)	RSD (%)
1	0	0.0861	undetectable	N/A	N/A
2	1.0 fM	0.2841	1.02 fM	96.8 %-102.9 %	3.8
3	10.0 fM	0.4002	9.82 fM	98.4 %-103.8 %	4.6
4	10.0 pM	0.7540	9.72 pM	97.5 %-102.3%	4.2

To evaluate the real application potential of our method, the developed protocol was used to determine the recovery rate of the miR-21 content in human serum samples (obtained from the 9th People's Hospital of Chongqing) with the standard addition method. Varying concentrations of miR-21 at 1.0 fM, 10.0 fM, 10.0 pM were spiked into the human serum samples diluted 10 times with 1×TAE/Mg buffer and the results were listed in Table S3. As we can see, the recoveries are in an acceptable range from 96.8 % to 103.8 % and the RSD is below 4.6 %, indicating that our developed approach can be applied to monitor miR-21 in complex serum media with a good accuracy.

REFERENCE

(1) C. F. Chen, D. A. Ridzon, A. J. Broomer, Z. H. Zhou, D. H. Lee, J. T. Nguyen, M. Barbisin, N. L. Xu, V. R. Mahuvakar, M. R. Andersen, K. Q. Lao, K. J. Livak and K. J. Guegler, *Nucleic. Acids. Res*., 2005, **33**, e179.

(2) H. M. Chan, L. S. Chan, R. N. S. Wong and H. W. Li, *Anal. Chem*., 2010, **83**, 6911-6918.

(3) D. M. Zhou, W. F. Du, Q. Xi, J. Ge and J. H. Jiang, *Anal. Chem*., 2014, **86**, 6763-6767.

(4) R. X. Duan, X. L.Zuo, S. T. Wang, X. Y. Quan, D. L. Chen, Z. F. Chen, L. Jiang, C. H. Fan and F. Xia, *J. Am. Chem. Soc*., 2013, **135**, 4604-4607.

(5) S. Su, J. W. Fan, B. Xue, L. H. Yuwen, X. F. Liu, D. Pan, C. H. Fan and L. Wang, *ACS Appl. Mater. Interfaces*, 2014, **6**, 1152-1157.

(6) Q. Xi, D. M. Zhou, Y. Y. Kan, J. Ge, Z. K. Wu, R. Q. Yu and J. H. Jiang, *Anal. Chem*., 2014, **86**, 1361-1365.

(7) Y. Q. Cheng, X. Zhang, Z. P. Li, X. X. Jiao, Y. C. Wang and Y. L. Zhang, *Angew . Chem. Int. Ed*., 2009, **121**, 3318–3322.

(8) H. F. Dong, J. Zhang, H. X. Ju, H. T. Lu, S. Y. Wang, S. Jin, K. H. Hao, H. W. Du, and X. J. Zhang, *Anal. Chem*., 2012, **84**, 4587−4593.