Supporting Information

An enzyme-free molecular catalytic device: dynamically self-assembled DNA dendrimers for in situ imaging of microRNAs in live cells

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Tables of Contents

1. Additional experimental section

Reagents and materials. N,N,N',N'-tetramethylethylenediamine (TEMED) and 4S Red Plus were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Acrylamide, bis-acrylamide and ammonium persulfate (APS) were ordered from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Healthy human serum was obtained from Jinjialing Community Health Service Station (Qingdao, China), and the healthy human serum was diluted 100 times with RNAase-free ultrapure water before use. All oligonucleotides used in this work were synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China). The sequences and secondary structures are listed in Table S1 and Fig. S1, respectively. The oligonucleotide stock solutions (0.1 mM) were prepared in RNAase-free ultrapure water, which were further diluted to a certain concentration with TE buffer (10 mM Tris-HCl, 1 mM EDTA-2Na, 12.5 mM $MgCl_2$, pH 8.0). The Y-scaffold DNA was prepared by annealing the mixture containing stoichiometric amounts of Y1, Y2, and Y3 (10 μM for each) at 95 ^oC for 5 min, and subsequently cooling to 25 ^oC at 0.1 ^oC/s for 2 h at least using a thermal cycler (LongGene Mini 1620, Hangzhou, China). Meanwhile, the DNA hairpins, H1, H2 and H3, were respectively annealed by the method described above to form the desirable secondary structures. All other reagents were of analytical grade and used without further purification. RNAase-free ultrapure water was used in all experiments.

Table S1. Oligonucleotide sequences used in this work

Strand	Sequence (from 5' to 3')
H1	CCCACCAACCAT-Dabcyl-CTAATCGTGATAGGGGTACAACACTAACCTTACCCCTATCACGATTAGCATTAA-FAM
H2	CACCAGACACACGGGGTACAACACTAACCTTTAATGCTAATCAGGTTAGTGTTGTACCCCTATCAC
H3	CCCTTCCTCTCGTAACCTTTAATGCTAATCGTGATAGGGGTAGATTAGCATTAAAGGTTAGTGTTG
Y1	ATGGTTGGTGGGTGGATCCGCATGACATTCGCCGTAAG
Y2	GTGTGTCTGGTGCTTACGGCGAATGACCGAATCAGCCT
Y3	CGAGAGGAAGGGAGGCTGATTCGGTTCATGCGGATCCA
mH1	CCCACCAACCAT-Dabcyl-CTAATCGTGATAGGGGTACAACACTAACCTTACCCCTATCACGATTAG**GTCAGC**-FAM
H1 (miR-21)	CCCACCAACCAC-Dabcyl-ATCAGACTGATGTTGACAACACAACCTTCAACATCAGTCTGATAAGCTA-FAM
H2 (miR-21)	CACCAGACACACGTTGACAACACAACCTTAGCTTATCAGAGGTTGTGTTGTCAACATCAGT
H3 (miR-21)	CCCTTCCTCTCGAACCTTAGCTTATCAGTAGTCAAGTTGCTGATAAGCTAAGGTTGTGTTG
Y1 (miR-21)	GTGGTTGGTGGGTGGATCCGCATGACATTCGCCGTAAG
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-155	UUAAUGCUAAUCGUGAUAGGGGU
one-base mismatched miR-155	UUAAUGCUAAU**G**GUGAUAGGGGU
three-base mismatched miR-155	UUAAU**A**CUAAU**G**GUGAUA**A**GGGU

Note: The mismatched bases are marked in bold and underlined.

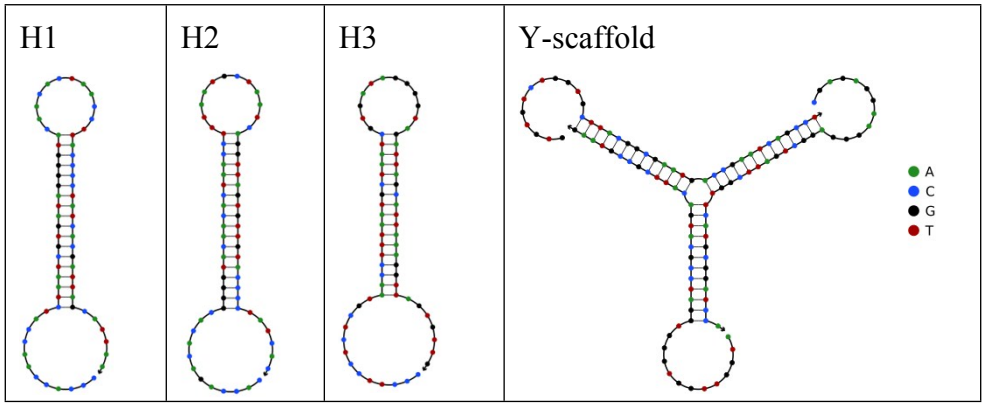

Fig. S1 Secondary structures of DNA hairpins and Y-scaffold DNA used in this work, which are predicted using the NUPACK software (www.nupack.org).

2. Zeta potential distribution of DNA dendrimers

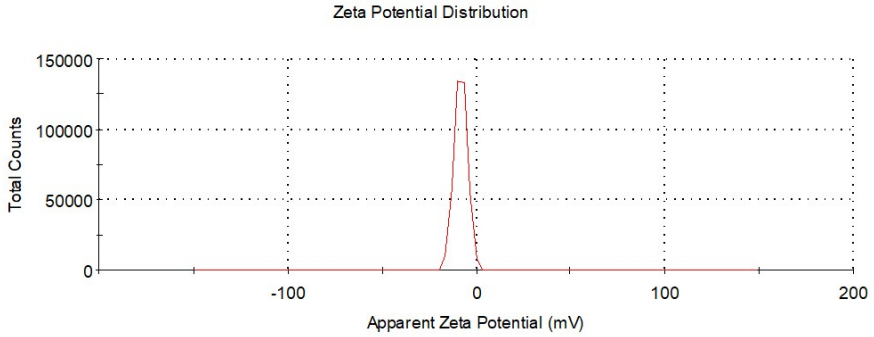

Fig. S2 Zeta potential of DNA dendrimers with the average value of -8.35 ± 3.44 mV.

3. Real sample assay

Table S2. Determination of miR-155 spiked in 100-fold diluted healthy human serum via bCHA

Sample no.	1	2	3
Added (nM)	5.0	25.0	50.0
Found (nM)	5.4	26.3	47.8
Recovery (%)	108.0	105.2	95.6
RSD (%)	3.2	3.3	2.8

4. Amplification efficiency of bCHA

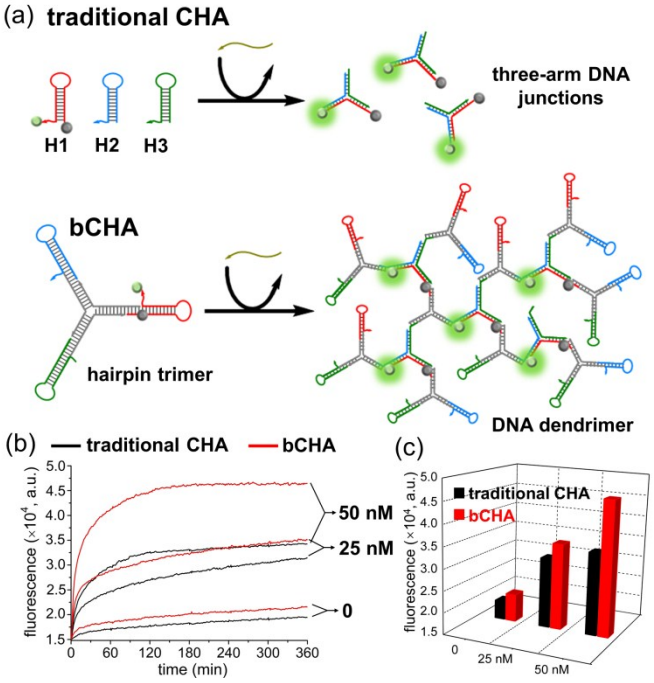

Fig. S3 Comparison of CHA and bCHA in response to different concentrations of initiator. (a) Schematic representation of CHA and bCHA. (b) Fluorescence kinetic curves of CHA and bCHA triggered by miR-155 with the concentrations of 0, 25 nM, and 50 nM, respectively. The concentration of hairpin trimers is 1.5 μM. (c) Fluorescence intensity derived from (b) at the reaction time of 360 min.

5. Stability test

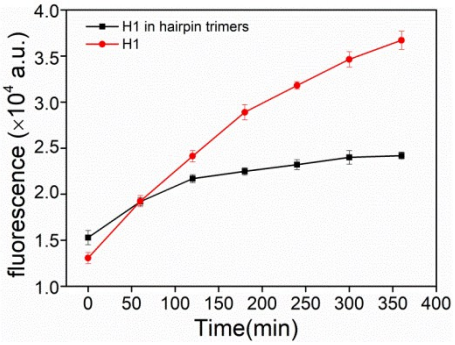

Fig. S4 Stability test of H1 in hairpin trimers and free H1 in 100-fold diluted healthy human serum. The error bars represent the standard deviation of three independent measurements. The concentrations of hairpin trimers and H1 are both 500 nM.

6. Mean fluorescence intensities from time course CLSM images

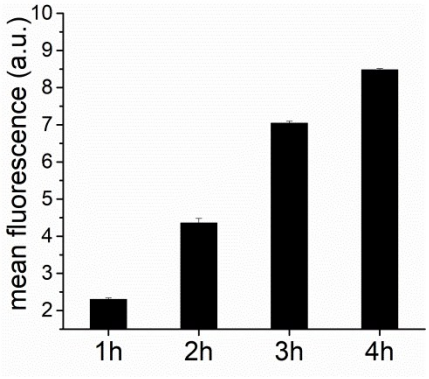

Fig. S5 Mean fluorescence intensities derived from time course CLSM images of in situ miR-155 analysis in MCF-7 cells via bCHA. MCF-7 cells are transfected with 200 nM hairpin trimers for 4 h at 37 °C. The error bars represent the standard deviation of three independent measurements.

7. Validation of the target-induced mechanism of bCHA in live cells

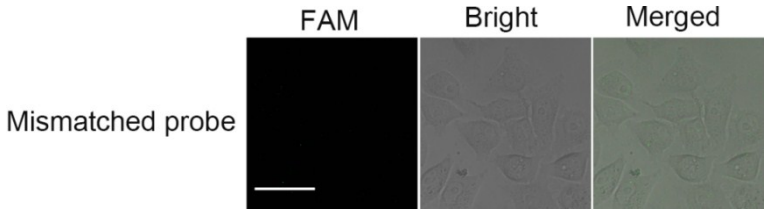

Fig. S6 CLSM images of MCF-7 cells which are incubated with hairpin trimers containing a mutated H1.

8. Mean fluorescence intensities from flow cytometric analysis

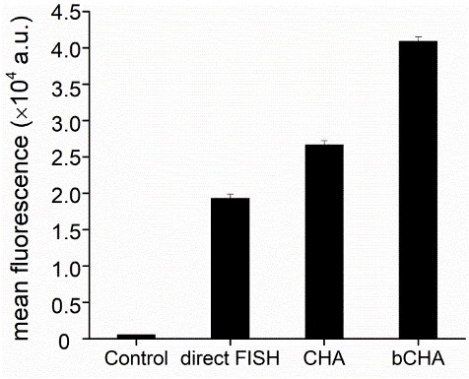

Fig. S7 Mean fluorescence intensities derived from flow cytometric analysis of MCF-7 cells after transfection of only H1, the mixture of hairpin monomers (H1, H2 and H3) and hairpin trimers at 37 °C for 4 h, respectively. The error bars represent the standard deviation of three independent measurements.

9. bCHA strategy for miR-21 detection

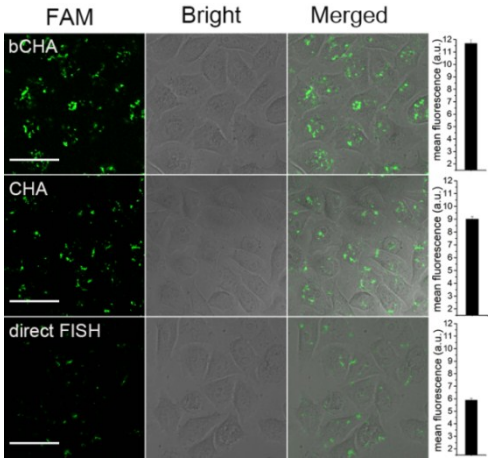

Fig. S8 The bCHA-based intracellular imaging of miR-21. Left: CLSM images of miR-21 in MCF-7 cells with the transfection of hairpin trimers (bCHA), the mixture of hairpin monomers (CHA) and only H1 (direct FISH) at 37 °C for 4 h, respectively. Scale bar: 20 μm. Right: mean fluorescence intensities derived from CLSM images. The concentration of each DNA strand is 200 nM. The error bars represent the standard deviation of three independent measurements.