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Accelerated DNA tetrahedron-based molecular beacon for efficient microRNA imaging in living cells

Chao Xing,*[a] Ziyi Chen,[b] Yuhong Lin,[b] Min Wang,[b] Xin Xu,[b] Junduan Dai,[b] Jun Wang*[a] and Chunhua Lu*[b]

a. Fujian Key Laboratory of Functional Marine Sensing Materials, Center for Advanced Marine Materials and Smart Sensors, Minjiang University, Fuzhou 350108, P. R. China. E-mail: chaoxing@mju.edu.cn; wangjun2@mju.edu.cn

b. MOE Key Laboratory for Analytical Science of Food Safety and Biology, Fujian College of Chemistry, Fuzhou University, Fuzhou 350116, P.R. China; E-mail: chunhualu@fzu.edu.cn

EXPERIMENTAL SECTION

Materials and Apparatus. All DNA sequences used in our experiments (Table S1) were synthesized by Sunya Biotechnology (Fuzhou, China). Cell Counting Kit-8 was purchased from Sigma-Aldrich (Shanghai, China); Cell culture products were purchased from Life Technologies (Carlsbad, CA). Exonuclease III was purchased from Thermo Fisher Scientific (Shanghai, China.). The fluorescence spectra were measured by a Cary Eclipse Fluorimeter (Varian Inc). Confocal microscopy imaging studies was operated on a NIKON-A1 laser-scanning confocal microscopy. Flow cytometric assay were measured by the FACSCanoto flow cytometer (BD Bioscience, U.S.A.). Ultrapure water (18.2 MΩ•cm) was used throughout this study. Unless specified, other chemicals involved in the experiment were analytical grade and used without further purification.

Construction of Accelerated-DTMB and uncombined HP-DTMB structure. The DTMB was self-assembled based on the previous protocol. Briefly, S1, S2, S3, and S4 were mixed with the equal molar ratio in 1× TAE/Mg buffer (20 mM Tris, 2 mM EDTA, 12.5 mM $MgCl_2$; pH 7.4). Then, the mixture was heated to 95 °C for 10 min, and then slowly cooled to room temperature at a speed of −1°C/min. Then, equimolar amounts of DTMB and HP were combined in 1× TAE/Mg buffer and incubated at 37 °C for 1h. The uncombined HP-DTMB structure was constructed by the direct mixing equimolar amounts of the DTMB with HP1 strand (HP without linker section, Table S1)

Characterization of the Accelerated-DTMB. The formation of DTMB and accelerated-DTMB was verified by 3% agarose gel electrophoresis and dynamic light scattering (DLS). For electrophoretic characterization, the sample-included gel was run in 1× TAE/Mg buffer at 80 V for 1 h and analyzed using an ChemiDocXRS System. The DLS characterization of the DTMB and accelerated-DTMB was carried out using diluted samples.

Fluorescence Measurements. The accelerated-DTMB nanoprobes were mixed with different concentrations of miR-21 in 200 μL 1× TAE/Mg reaction buffer, and incubated at room temperature for 60 min. Next, the fluorescence of FAM was collected on a Cary Eclipse Fluorimeter between 500 and 650 nm by use of excitation wavelength at 488 nm. For comparison of the amplification effects, the fluorescence testing of the uncombined HP-DTMB was performed by mixing HP and DTMB with targets under the same conditions. To compare the accelerating ability the of the accelerated-DTMB, the real-time fluorescence signal of the accelerated-DTMB and traditional CHA with miR-21 of 20 nM were measured every 3 minutes for 2 hours.

Cell Culture and Confocal Fluorescence Imaging. For confocal microscopy assay, HeLa, MCF-7 and L02 cells cells lines were separately cultured in 1640 medium with 10% fetal calf serum and cultured overnight

at 37 °C in a 5% CO_2 atmosphere. Then, after addition of DNA nanoprobes (100 nM), the cells were cultured for the desired time at 37 °C. Subsequently, the samples were washed with PBS for three times to remove free probes and those nonspecifically attached on cellular surfaces. Finally, fluorescence imaging of living cells was carried out on a laser-scanning confocal microscopy system with different laser transmitter.

Table S1. DNA sequences in this work.

Name	Sequence (5'-3')
S1	CCGACGTAGCTGTGTTTTTTTTTTTTAGTCTGAATTCCTGGAGATA CATGGCATTTGCTACACGCCCTATTAGAAGGTCCGATTTAAGCT/FAM/ AGAGCATCCAATGTAGCTTATCAGACTGCATTGGATGCT/Dabcyl/CCGA AGAGCCGTAGCA
S2	GCAGTTGACGCGACAGTCGTTCAAGCCTTTCGGACCTTCTAATAG GGCGTGTAGCATTATGCGAGGGTCCAATACTCTGTTCCGG
S3	GGCTTGAACGACTGTCGCGTCAACTGCTTACGACACTACGTAACG GTCGAGGACTGTTGCTACGGCTCTTCG
S4	TGCCATGTATCTCCAGGAATTCAGACTTTCAGTCCTCGACCGTTA CGTAGTGTCGTTTCCGGAACAGAGTATTGGACCCTCGCAT
HP	CACAGCTACGTCGGTTTCAACATCAGTCTGATAAGCTACATTGGA TGCTCTAGCTTATCAGACTG
HP1	CAACATCAGTCTGATAAGCTACATTGGATGCTCTAGCTTATCAGA CTG
MBs	TAAGCT/FAM/AGAGCATCCAATGTAGCTTATCAGACTGCATTGGATGC T/Dabcyl/C
miRNA21	UAG CUU AUC AGA CUG AUG UUG A
miR-21 mimics	U*A*G*CUUAUCAGACUGAUGUU*G*A*
Anti-miR-21	U*C*A*ACAUCAGUCUGAUAAGC*U*A*
miR-429	UAAUACUGUCUGGUAAAACCGU
miR-200b	UAAUACUGCCUGGUAAUGAUGA
let-7d	AGAGGUAGUAGGUUGCAUAGUU

Table S2. Comparison of different sensor systems for miRNA detection.

System	linear range	Sensitivity	Ref.
AuNP Loaded Split-DNAzyme Probe	0-1 nM	10 pM	1
DNA Triangle-Protected Molecular Beacon	0.3-50 nM	100 nM	2
DNAzyme-based amplification strategy	0.1-10 nM	44 pM	3
Spectral Crosstalk Correction	0.02-10 nM	11pM	4
CHA-HCR DNA circuit	0-0.5nM	2 pM	5
accelerated-DTMB	0-8nM	2 pM	This work

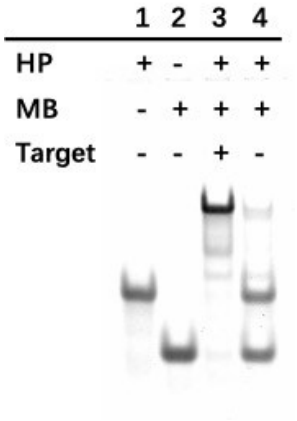

Figure S1. PAGE image of the catalytic hairpin assembly reaction.

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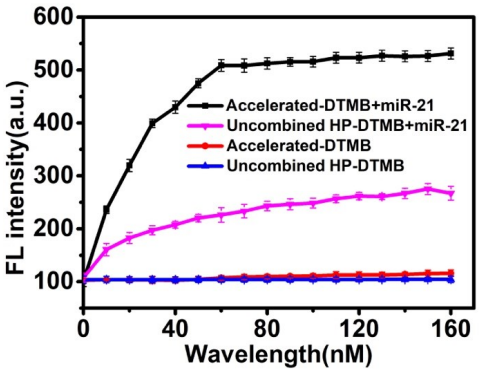

Figure S2. Real-time monitoring of fluorescence signals of different systems in response to 20 nM miR-21.

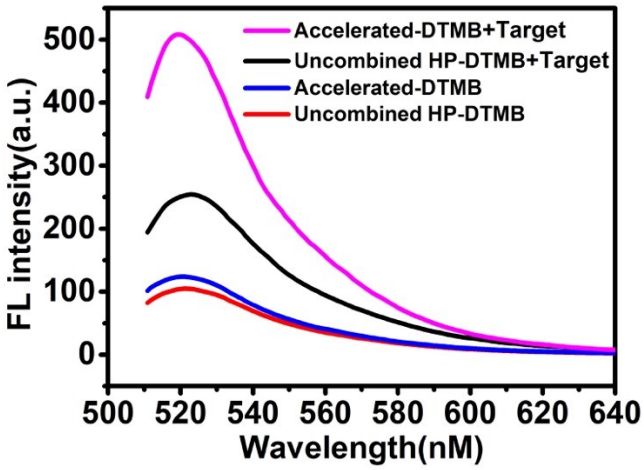

Figure S3. Fluorescence emission spectra of uncombined HP-DTMB and accelerated-DTMB responds to DNA targets in vitro at 37 °C with 488 nm excitation wavelength. The concentrations of probe and target were 100 nM and 20 nM, respectively.

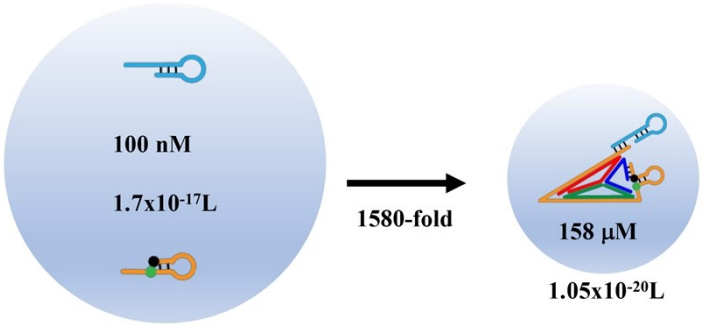

Figure S4. Comparison of the reaction area and local concentration of two hairpin probes for uncombined HP-DTMB and accelerated-DTMB.

We used the equation V = 1/cN to estimate the volume of the reaction sphere using the concentrations of the probes[6], in which c is the concentration of the hairpin probe, and N represents the Avogadro constant. For the uncombined HP-DTMB with 100 nM each of HP and MB, the volume of the sphere containing DNA hairpin probe was calculated to be 1.7×10^{-17} L with a radius of 157 nm. For the accelerated-DTMB, the distance between the two hairpin probes was about 13.6 nm (40 bp, maximum length) and the local concentrations was calculated to be 158 μM, which was increased 1580-fold compared to the reaction in the bulk condition. Because the reaction

speed is highly related with collision frequency, the increased local concentration of HP and MB significantly enhanced the collision frequency of two hairpin probes, causing improved reaction time and enchanted catalytic efficiency.

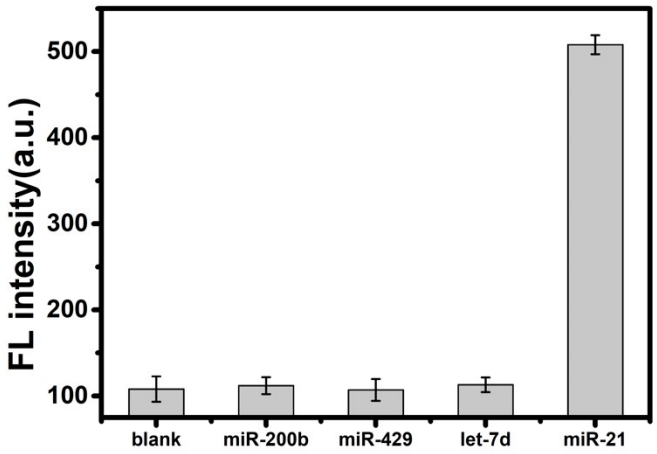

Figure S5. Specificity of the accelerated-DTMB for several miRNA targets. Error bars were estimated from three replicate measurements.

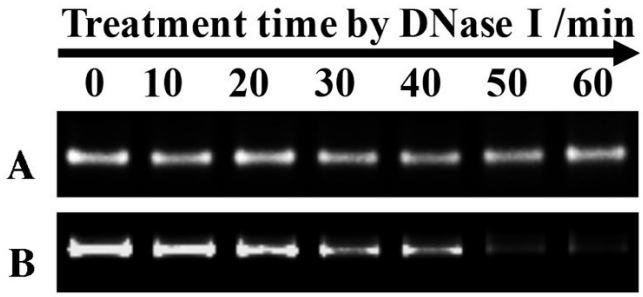

Figure S6. Electrophoresis characterization for the degradation of accelerated-DTMB (A) and general MBs (B) treated with 0.5U/mL DNase I.

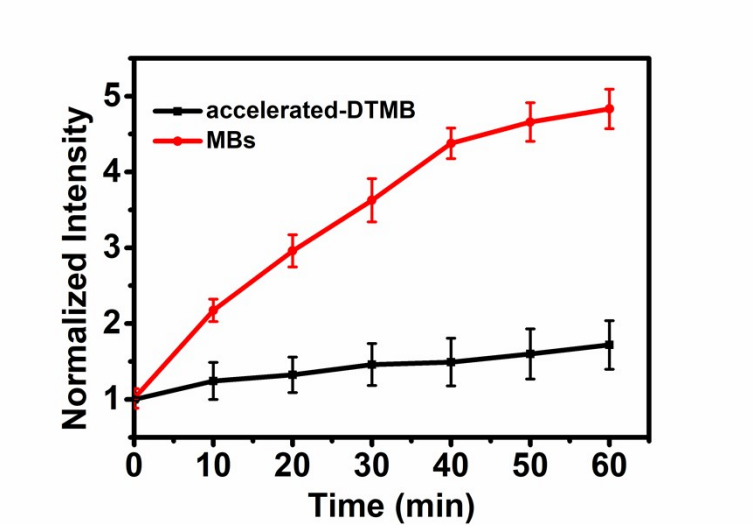

Figure S7. The stability analysis of accelerated-DTMB and general MBs in 0.5U/mL DNase I respectively. Error bars indicated SDs across three repetitive assays.

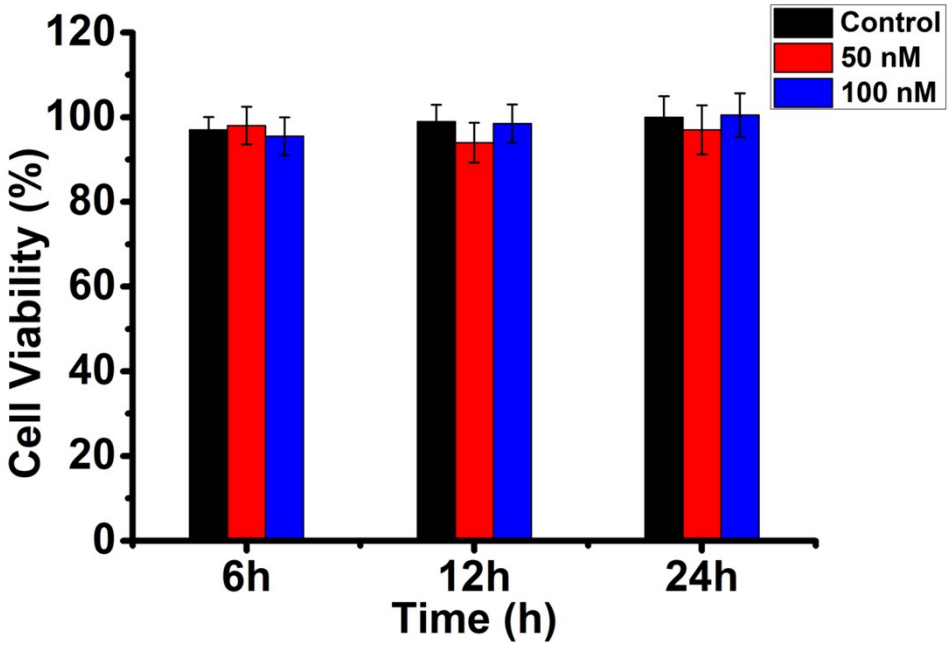

Figure S8. Cytotoxicity of the accelerated-DTMB incubated with HeLa cells at different concentrations of probes.

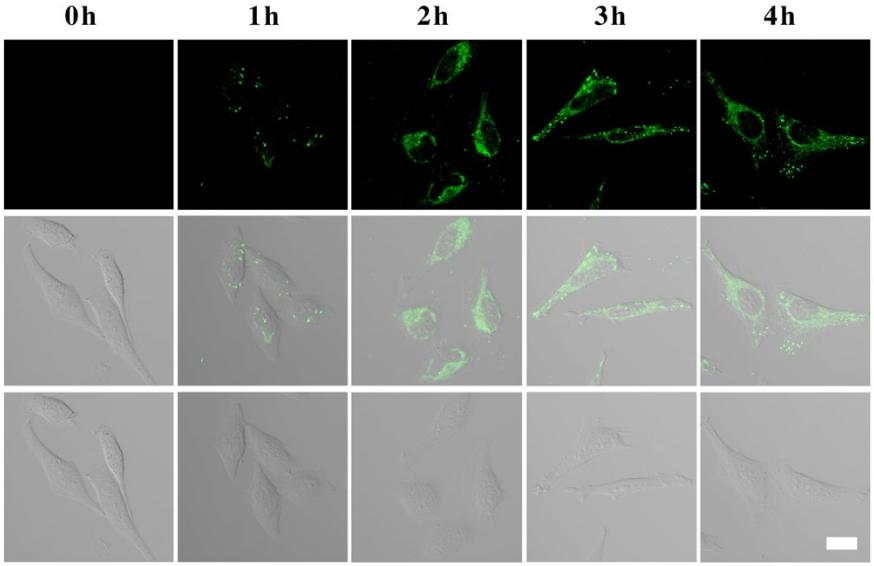

Figure S9. Optimization of incubation time for accelerated-DTMB in living cells. Hella cells were incubated with accelerated-DTMB for different time points at 37 °C for confocal microscopy. Scale bars are 20 μm.

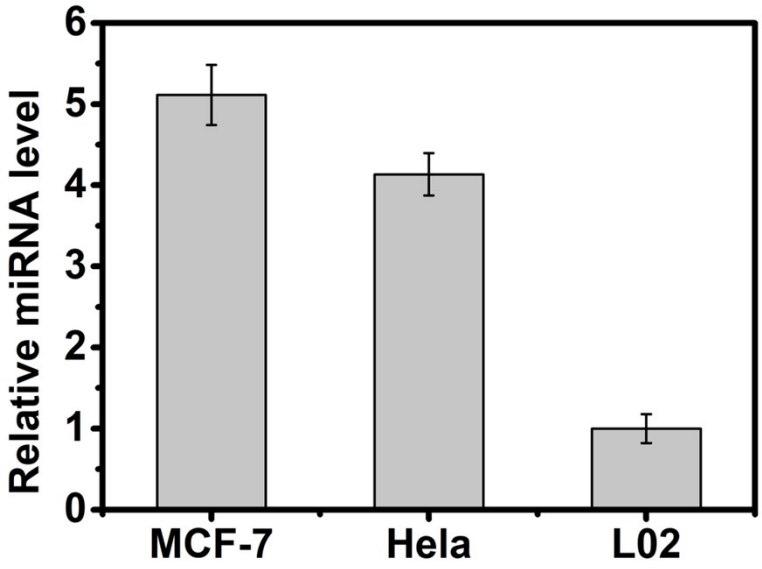

Figure S10. qRT-PCR analysis of relative expression levels of miR-21 in L02 cells, MCF-7 cells and HeLa cells.

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