Electronic Supporting Information (ESI)

Sensitive RNase A detection and intracellular imaging using natural compound-assisted tetrahedral DNA nanoprobe

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EXPERIMENTAL SECTION

Chemicals and Materials

All HPLC-purified oligonucleotide strands and RNase A were provided by Takara Biotechnology Co., Ltd. (Dalian, China). The sequences of these oligonucleotides are shown in Table 1. RNase A, RNase H, T4PNK, T4 DNA Ligase and bovine serum albumin (BSA) were purchased from Takara Biotechnology Co. Ltd (Dalian, China). UDG and APE1 were purchased from New England Biolabs LTD (Beijing NEB). Fpg was purchased from New England Biolabs Inc. (Beverly, Mass, USA). All aqueous solutions used in the experiments were prepared using ultrapure water (≥18MΩ, Milli-Q water purification system, Millipore). Human liver hepatocellular carcinoma cell line HepG2, Human umbilical vein endothelial cells HUVECs and Vascular smooth muscle cells VSMCs were kept in our lab.

Instrumentation

All fluorescent spectra were measured using F-2500 fluorescence spectrometer (Hitachi Company, Tokyo, Japan). The excitation wavelength was 450 nm with an emission range of 494-580 nm. The fluorescence intensity at 521 nm was used to evaluate the performance of the proposed assay. Both of the emission and excitation slit widths were set at 10 nm. The software Sigmaplot 12.0 was used to process experimental data. Cells were cultured in a Thermo FORMA 3111 CO_2 incubator (Thermo Fisher, USA). Cell imaging was performed using an Olympus IX-70 inverted microscope with an Olympus FV 500 confocal scanning system (Japan).

Synthesis of Tetrahedral DNA Nanostructures

TDN were synthesized as previously reported.[1, 2] L1, L2, L3, and L4 were mixed in the TM buffer (10 mM Tris-HCl, 50 mM $MgCl_2 \cdot 6H_2O$, pH 8.0) with same concentrations. The mixture was denatured at 95℃ for 10 min and then rapidly cooled to 4℃ for 35 min. The assembled TDN was allowed to store at 4℃ for at least 1 week.

Optimization of the reaction time

To optimize the time of enzymatic reacted with the substrate, L1 (final concentration 200 nM) and RNase A (0.4 ng μL^{-1}) were firstly mixed in a 50 μL standard reaction system and incubated for 0, 10, 20, 30, 40, 50, 60 and 75 min at 37℃. followed by the addition of L2, L3 and L4 with a final concentration of 200 nM. The fluorescence signal intensities were monitored after the resulting mixture was heated to 95℃ for 10 min and cooled to 4℃ for 35 min.

Optimization of the reaction temperature of RNase A

In order to optimize the temperature of the enzyme reaction, the mixture of L1 (final concentration 200 nM) and RNase A (0.4 ng μL^{-1}) were firstly incubated at different temperatures（20℃ , 30℃ , 37℃ , 45℃ and 55℃) in a 50 μL standard reaction system, followed by the addition of L2, L3 and L4 with a final concentration of 200 nM. The fluorescence signal intensities were monitored after the resulting mixture was heated to 95℃ for 10 min and cooled to 4℃ for 35 min.

Sensitivity of DNA Tetrahedron-Based Method

First, 1 μL of L1 (200 nM) and different concentrations of RNase A were added to a reaction system with total volume of 50 μL, and incubated at 37℃ for 60 min. Thereafter, L2, L3 and L4 (200 nM) were added and incubated at 95℃ for 10 min, and finally rapidly cooled to incubate at 4℃ for 35 min. Finally, the fluorescence intensity was measured at Ex/Em = 450/521 nm.

Specificity analysis

For the RNase A specificity assay, 1 U of UDG, APE1, RNase H, T4 PNK, Fpg, T4 DNA Ligase and 40 ng BSA instead of RNase A was added to the 50 μL buffer containing L1 (final concentration 200 nM) and incubated for 60 min at 37℃, followed by the addition of L2, L3 and L4 (final concentration of 200 nM), the resulting mixture was heated to 95℃ for 10 min and cooled to 4℃ for 35 min. The fluorescence intensities of the samples were measured as described previously.

Natural compounds screening

Compounds were diluted with 100% DMSO to 500 μM. For compounds screening, after the mixture of RNase A and drug solution were incubated at 37℃ for 10 min, L1 was added into the mixture and continued to react for 60 min. Then, the L2, L3, and L4 strands were sequentially added. The mixture was heated at 95℃ for 10 min, then rapidly cooled to 4℃ for 35 min, and subjected to fluorescence measurement as described above. The detail information of natural compounds were listed in Table S2.

Growth inhibition assay (MTT)

To investigate the cytotoxicity of nature compounds, MTT assay was carried out when nature compounds existed. HepG2 cells were dispersed with replicate 96-well microtiter plates at a density of 1×10^6 cells/well. Plates were then maintained at 37°C in 5% CO_2 atmosphere for 24 h. Thereafter, the cells were treated with varying concentrations of nature compounds (0, 10, 20μM) for 4 h and 100 μL MTT solutions were then added to each well for 4 h. After removing the remaining MTT solution, 100 μL DMSO was added to each well to dissolve the formazan crystals. The absorbance was measured at 490 nm with microplate reader. Human umbilical vein endothelial cells HUVECs and Vascular smooth muscle cells VSMCs were measured in the same way as HepG2 cells.

enzymatic resistance assay of nanoprobe

To demonstrate the nucleic acid stability of the TDN, different concentrations of DNase I were incubated with the synthetically finished tetrahedron for 30 min at 37℃. Then, the mixture was run through PAGE in 120 V of 1 x TBE buffer for 1 h.

Confocal fluorescence imaging

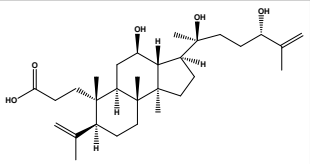
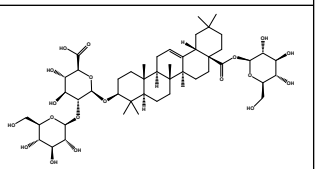
In the experiment of RNase A expression level, HepG2 cells were seeded on the 12-well plate with a density of 5×10^5 cells/well, then washed two times with PBS buffer, HepG2 cells were then treated for 2 h with four natural drugs (G-10, Chi V, CY-21, CY-24) and RNase A inhibitors (with a final concentration of 20 u/ml), respectively. In addition, a group of HepG2 cells without any Drug was used as a control. After 2 hours, the cells were washed three times with PBS buffer, TDN (final concentration 200 nm) were added, and cultured at 37℃ for 4 h. Prior to imaging, the cells on the slides were washed three times with PBS (pH=7.4) buffer to remove nano-probes that were not adsorbed by the cells, and the fluorescence emission was collected by the multi-dimensional live cell imaging system OLYMPUS FV1200 and imaged with an oil immersion objective 60×.

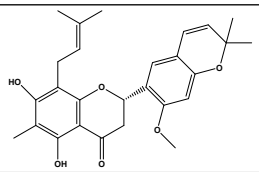
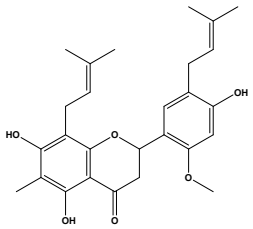
Supporting tables

Table S1. Sequences of Oligonucleotides Used in This Work.

Oligo	Sequences (5' to 3')
L1	(**FAM**)-ATTTArUCACCCGCCATAGTAGACGTATCACCAGGCAGTTGAGACGAACATTCCTAAGTCTGAA-(**BHQ1**)
L2	ACATGCGAGGGTCCAATACCGACGATTACAGCTTGCTACACGATTCAGACTTAGGAATGTTCG
L3	ACTACTATGGCGGGTGATAAA-**(BHQ1)**-ACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCC
L4	ACGGTATTGGACCCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCG

Table S2. Natural Compounds

Compound name	Molecular formula	Chemical Name	Structure
CYJ-G-10 (G-10 or G10)	$C_{30}H_{50}O_5$	(12R,20S,24S)-12,20,24-trihydroxy-3,4-*seco*-dammara4(28),25-dien,3-oic acid	
Chikusetsusaponin V (Chi V)	$C_{48}H_{76}O_{19}$	β-D-Glucopyranosiduronic acid, (3β)-28-(β-D-glucopyranosyloxy)-28-oxoolean-12-en-3-yl 2-O-β-D-glucopyranosyl	

CY-21	$C_{27}H_{30}O_6$	Tripteryol A	
CY-24	$C_{27}H_{32}O_6$	((2S)-5,7,4'-trihydroxy-2'-methoxy-8,5'-di(3-methyl-2-butenyl)-6-methylflavanone	

Supporting Figures

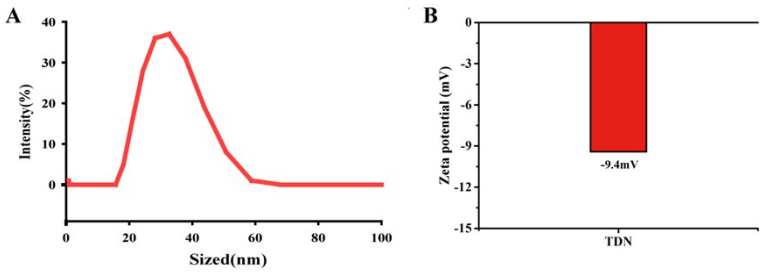


Fig. S1. (A) The size distribution of TDNs. **(B)** The zeta potential of TDNs. Data are presented as mean ± SD (n = 4).

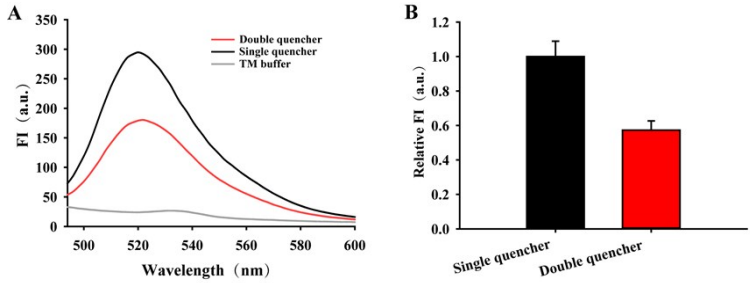


Fig. S2. (**A)** The wavelength scan of different samples. **(B)** The relative fluorescence intensity between the single quenching and the double. [L1] = [L2] = [L3] = [L4] = 200 nM.

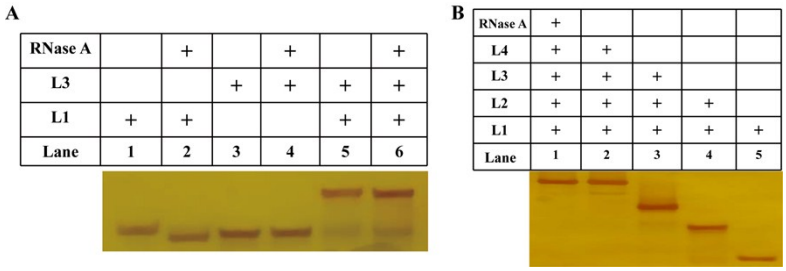


Fig. S3. (A) The result of PAGE electrophoresis with RNase A. **Lane 1**: L1; **Lane 2**: L1 + RNase A (200 ng μL^{-1}); **Lane 3**: L3; **Lane 4**: L3 + RNase A (200 ng μL^{-1}); **Lane 5**: L1 + L3; **Lane 6**: L1 + L3 + RNase A (200 ng μL^{-1}); [L1] = [L3] = 200 nM. **(B)** Electrophoretic analysis of synthetic tetrahedral products with or without RNase A. **Lane 1**: L1 + L2 + L3 + L4 + RNase A (200 ng μL^{-1}); L1; **Lane 2**: L1 + L2 + L3 + L4; **Lane 3**: L1 + L2 + L3; **Lane 4**: L1 + L2; **Lane 5**: L1; [L1] = [L2] =

[L3] = [L4] = 200 nM.

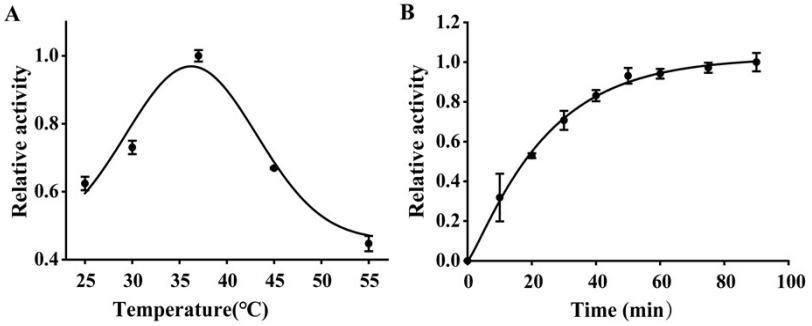


Fig. S4. The optimization of assay conditions. **(A)** The fluorescence intensity change with different temperature. **(B)** The fluorescence intensity change at different time point. [L1] = [L2] = [L3] = [L4] = 200 nM, [RNase A] = 0.4 ng μL^{-1}.

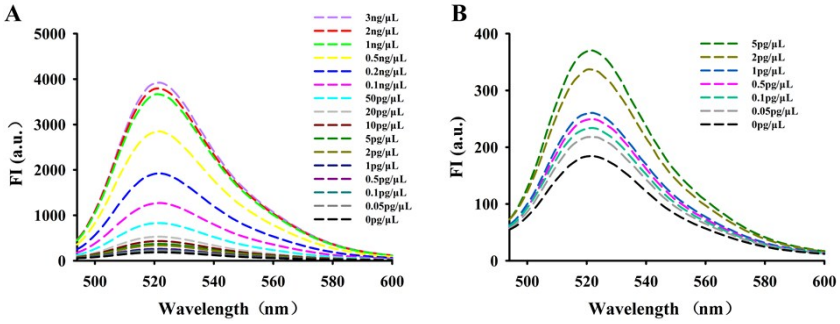


Fig. S5. (A) Fluorescence emission spectra of the method responses to various concentration of RNase A. **(B)** Fluorescence spectrum of low concentration. [L1] = [L2] = [L3] = [L4] = 200 nM. Ex/Em = 450/521 nm. Error bars SD, n = 3.

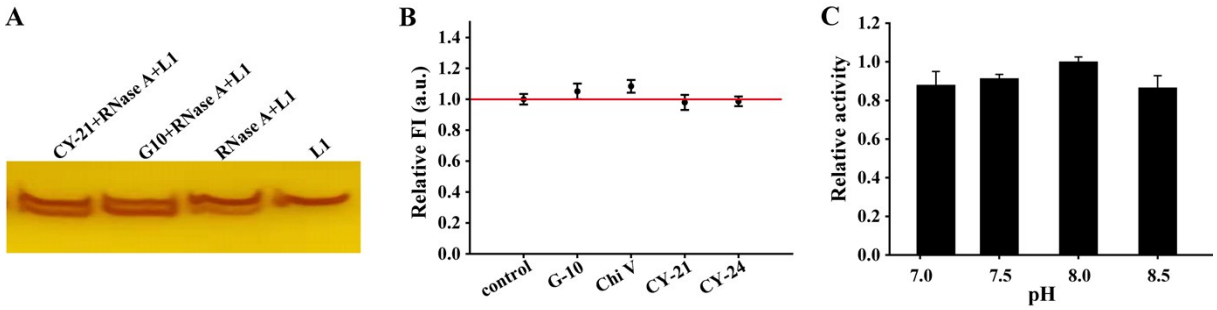


Fig. S6. (A) The result of PAGE electrophoresis with drug and RNase A. [RNase A] = 0.2 ng μL^{-1}. [L1] = 200 nM, [RNase A] = 0.2 ng μL^{-1}. [G10] = [CY-21] = 20 μM. **(B)** The fluorescence intensity of TDNs in TM buffer (which contains different natural compounds and does not contain RNase A). **(C)** The graphical presentation of the effects of natural compounds on the RNase A activity under different pH conditions. [L1] = [L2] = [L3] = [L4] = 200 nM. [RNase A] = 0.2 ng μL^{-1}. [G10 = 20 μM]. Ex/Em = 450/521 nm. Error bars SD, n = 3.

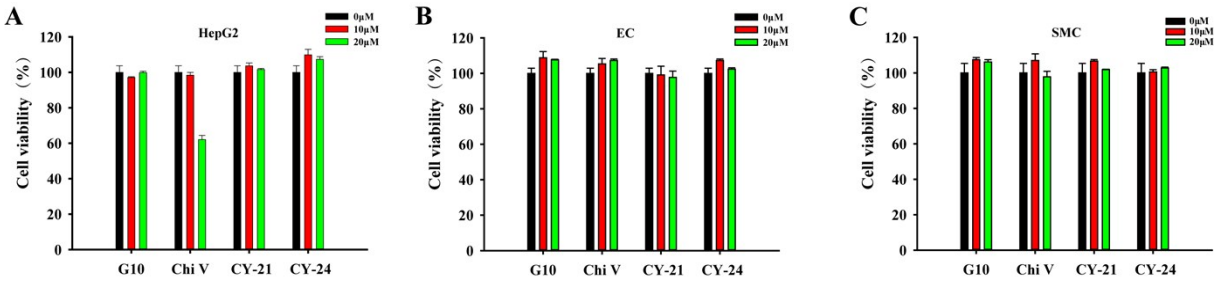


Fig. S7. Cell viability assay. **(A)** Cell viability of HepG2 cells after incubating with different native compounds for 4 h. **(B)**

Cell viability of HUVE cells after incubated with different native compounds for 4 h. **(C)** Cell viability of VSM cells after incubated with different native compounds for 4 h.

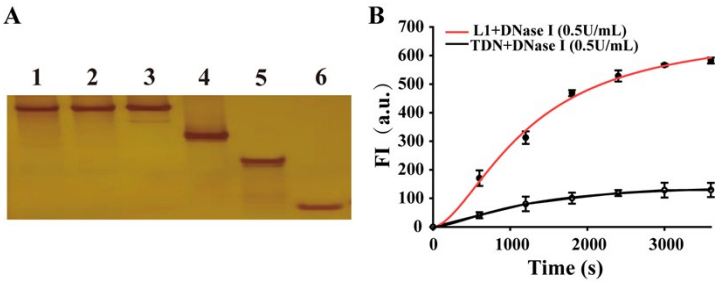


Fig. S8. (A) PAGE analysis. **Lane 1**: L1 + L2 + L3 + L4 + DNase I (final concentration of 0.5 U mL^{-1}); **lane 2**: L1 + L2 + L3 + L4 + DNase I (final concentration is 0.35 U mL^{-1}); **lane 3**: L1 + L2 + L3 + L4; **lane 4**: L1 + L2 + L3; **lane 5**: L1 + L2; **lane 6**: L1. [L1] = [L2] = [L3] = [L4] = 200 nM. **(B)** The resistant ability investigation of TDN and L1 strand to DNase I digestion.

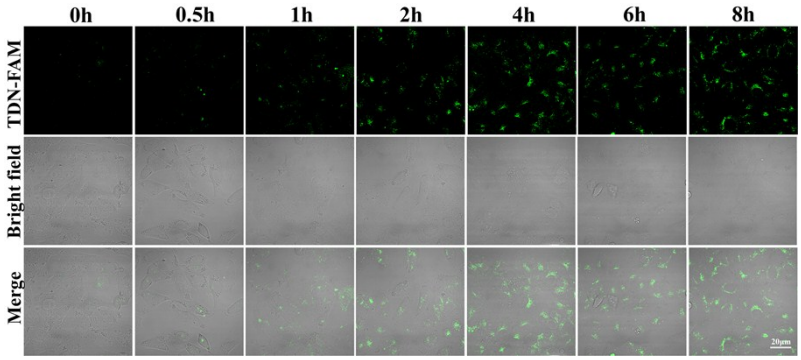


Fig. S9. Optimization of incubation time for tetrahedron with living cells. [TDN] = 200 nM. Scale bars are 20 μm.

References

1. X. Shao, S. Lin, Q. Peng, S. Shi, X. Wei, T. Zhang and Y. Lin, *Small*, 2017, **13**, 1602770.
2. Q. Peng, X.-R. Shao, J. Xie, S.-R. Shi, X. Wei, T. Zhang, X. Cai and Y. Lin, *Acs App. Mater. Interfaces*, 2016, **8**, 12733-12739.