Supporting Information for

Label-free and enzyme-free signal amplification strategy for the sensitive RNase H activity assay

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Table S1 Comparison of this method with the previous fluorometric/colorimetric methods.

Key components or methods	Signal	Detection limit (U/mL)	Limitations	Reference
Dual-labeled molecular beacon probe	Fluoro metric	15	Modification with fluorophore and quencher	1
Dual-pyrene-labeled beacon	Fluoro metric	5	Dual-modification with pyrenes	2
G-quadruplex/NMM complex	Fluoro metric	0.2	-	3
Gold nanoparticle (AuNP)-RNA-fluorescent dye conjugate	Fluoro metric	0.0043	• Modification of RNA with fluorophore • Pre-modification of AuNPs with RNA-fluorescent dye conjugate	4
Unmodified AuNPs	Colori metric	-	Poor stability of AuNPs against salt conditions in enzymatic reaction buffers	5
Target-triggered CHA producing G-quadruplex/NMM complexes	Fluoro metric	0.037	-	This work

Table S2 Nucleic acid sequences employed in this work.

Strand name	**Nucleic acid sequence (5' → 3')[a], [b]**
B-RNA	uau agu ccg ugu gac u
T-DNA	AGT CAC ACG GAC TAT A
HP1	GGG TTT TTT ATA GTC CGT GTG ACT ATG GAC TAT AGG AGT CAC ACG GGC GGG TAG GG
HP2	GGG TTT TTT GTG TGA CTC CTA TAG TCC ATA GTC ACA CGG ACT ATA GGG CGG GTA GGG

[a] DNA sequence is written in capital letters and RNA sequence is written in small letters.

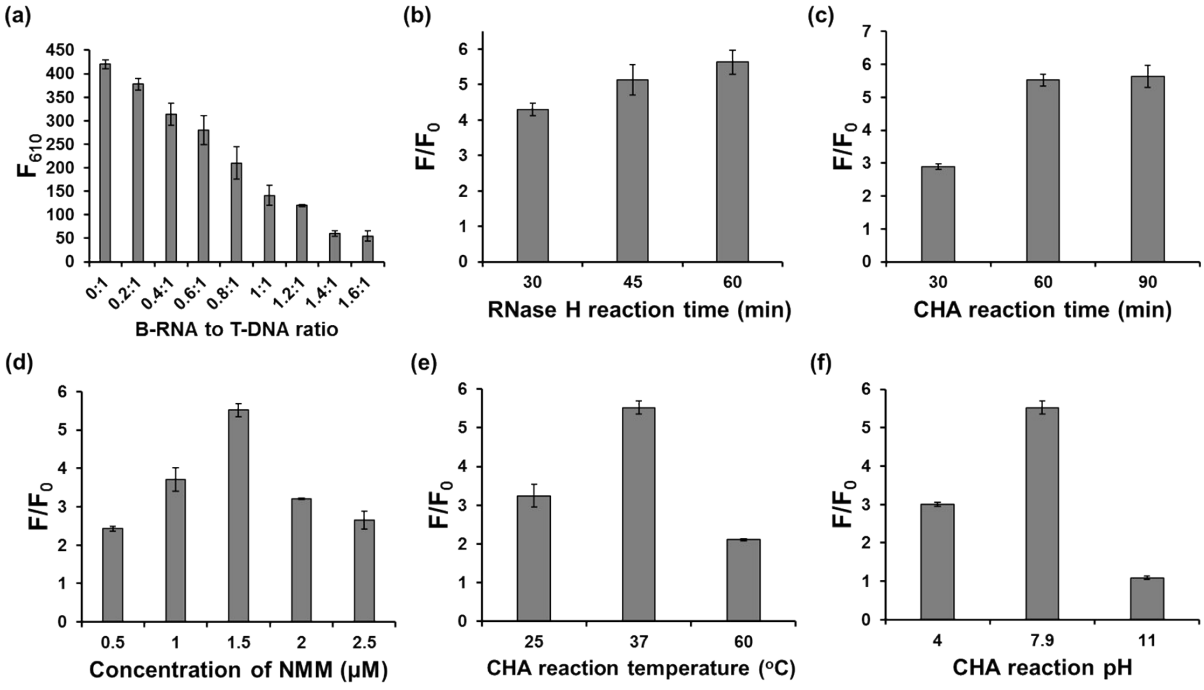
[b] The colors of nucleic acid sequences correspond to those in Scheme 1.

Table S3 ΔG of hybridized complexes or structures, where ΔG is the Gibbs free energy change by hybridization.

Hybridized complex or structure	ΔG (kcal/mol)
Hairpin structure of HP1	-12.78
Hairpin structure of HP2	-10.29
T-DNA/HP1 complex	-25.7
T-DNA/HP2 complex	-11.11

As shown in Table S3, ΔG for T-DNA/HP2 complex (-11.11 kcal/mol) is comparable to that for hairpin structure of HP2 (-10.29 kcal/mol) whereas ΔG for T-DNA/HP1 complex (-25.7 kcal/mol) is negatively larger than that for hairpin structure of HP1 (-12.78 kcal/mol). When considering that negatively larger ΔG implies more favorable reaction, these results clearly indicate that T-DNA preferentially hybridizes with HP1 to effectively initiate the CHA reaction, not HP2.

Fig. S1 Optimization of the reaction conditions for the efficient assay of RNase H activity. (a) Optimization of the B-RNA to T-DNA ratio for the lowest background signal. Fluorescence intensities at 610 nm from NMM with different B-RNA to T-DNA ratios. The CHA reaction time, NMM concentration, CHA reaction temperature, and CHA reaction pH are 90 min, 1.5 μM, 37 ^{o}C, and 7.9, respectively. (b) Optimization of the RNase H reaction time. The B-RNA to T-DNA ratio, CHA reaction time, NMM concentration, CHA reaction temperature, and CHA reaction pH are 1.4:1, 90 min, 1.5 μM, 37 ^{o}C, and 7.9, respectively. (c) Optimization of the CHA reaction time. The B-RNA to T-DNA ratio, RNase H reaction time, NMM concentration, CHA reaction temperature, and CHA reaction pH are 1.4:1, 60 min, 1.5 μM, 37 ^{o}C, and 7.9, respectively. (d) Optimization of the NMM concentration. The B-RNA to T-DNA ratio, RNase H reaction time, CHA reaction time, CHA reaction temperature, and CHA reaction pH are 1.4:1, 60 min, 60 min, 37 ^{o}C, and 7.9, respectively. (e) Optimization of the CHA reaction temperature. The B-RNA to T-DNA ratio, RNase H reaction time, CHA reaction time, NMM concentration, and CHA reaction pH are 1.4:1, 60 min, 60 min, 1.5 μM, and 7.9, respectively. (f) Optimization of the CHA reaction pH. The B-RNA to T-DNA ratio, RNase H reaction time, CHA reaction time, NMM concentration, and CHA reaction temperature are 1.4:1, 60 min, 60 min, 1.5 μM, and 37 ^{o}C, respectively. F_0 and F are the fluorescence intensities at 610 nm from NMM in the absence and presence of RNase H, respectively. The final concentration of RNase H is 30 U/mL.



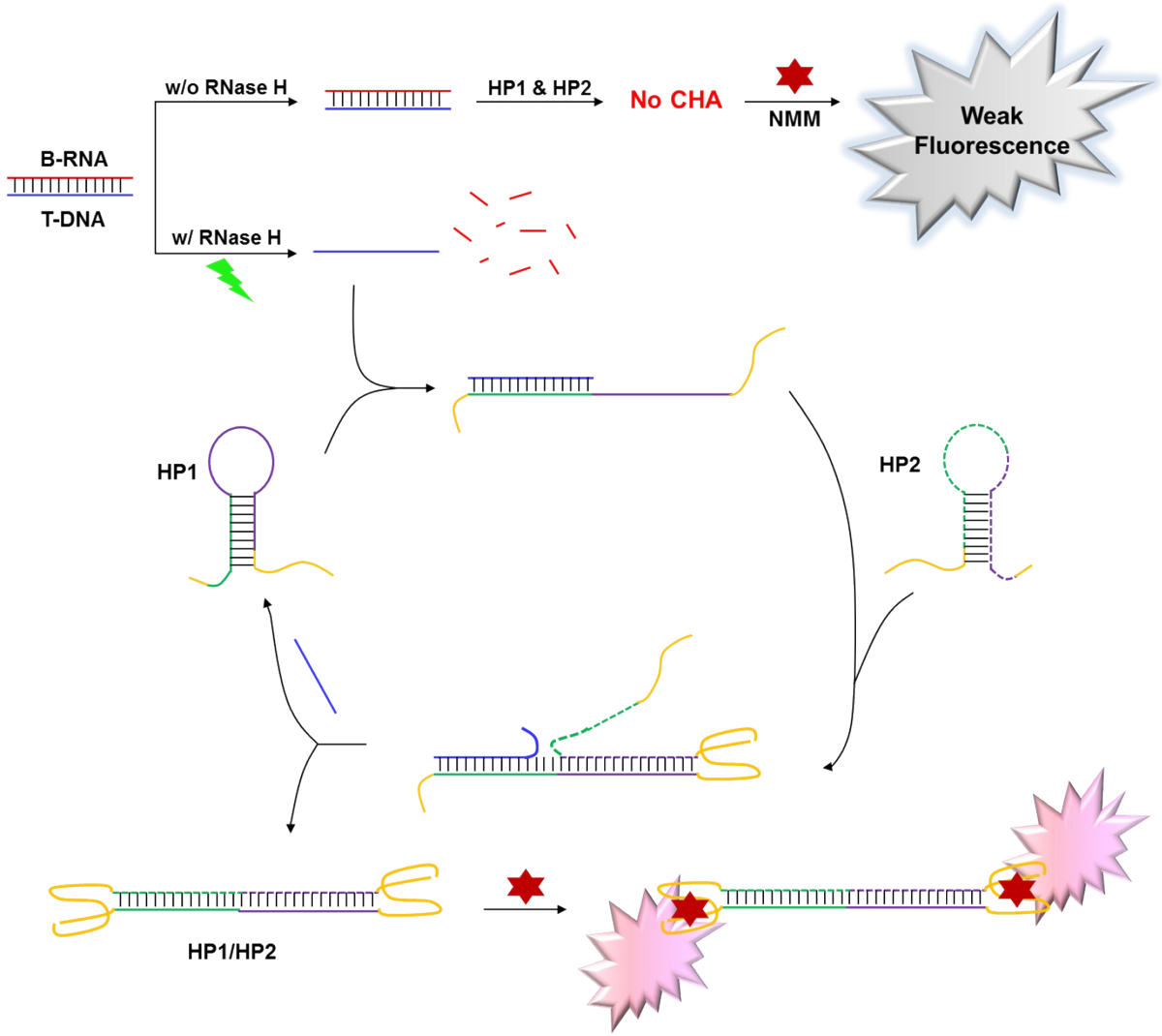


Fig. 1 (Enlarged version) Schematic illustration of the RNase H activity assay based on the target-triggered CHA producing a large number of G-quadruplexes with the significant fluorescence enhancement from NMM.

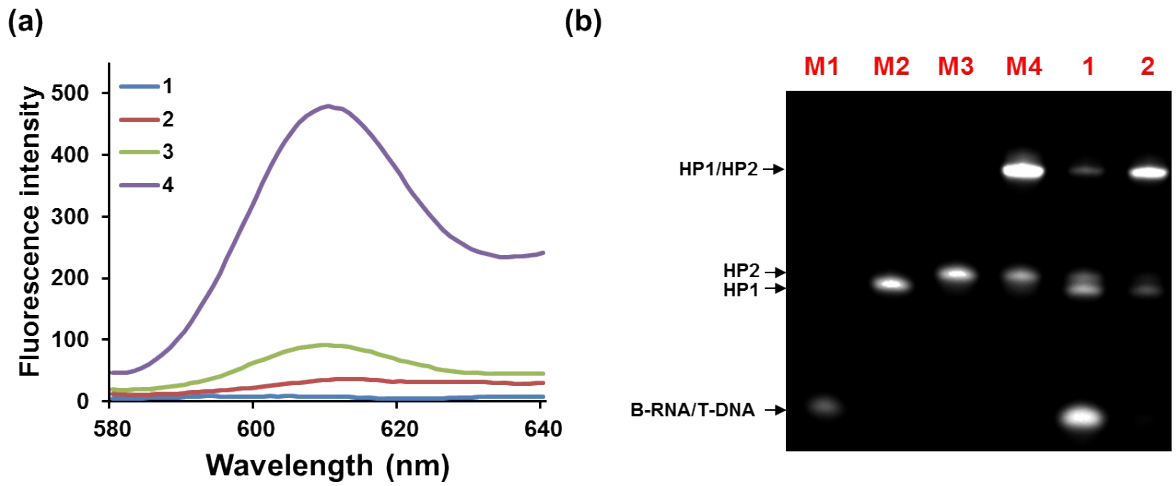


Fig. 2 (Enlarged version) Feasibility of the RNase H activity assay. (a) Fluorescence emission spectra from NMM in the different samples (1: B-RNA/T-DNA, 2: B-RNA/T-DNA+RNase H, 3: B-RNA/T-DNA+HP1+HP2, 4: B-RNA/T-DNA+HP1+HP2+RNase H). (b) Polyacrylamide gel electrophoresis image of products after the target-triggered CHA (1: B-RNA/T-DNA+HP1+HP2, 2: B-RNA/T-DNA+HP1+HP2+RNase H). Lanes M1, M2, M3, and M4 show the markers for B-RNA/T-DNA, HP1, HP2, and HP1/HP2, respectively. The final concentration of RNase H is 30 U/mL.

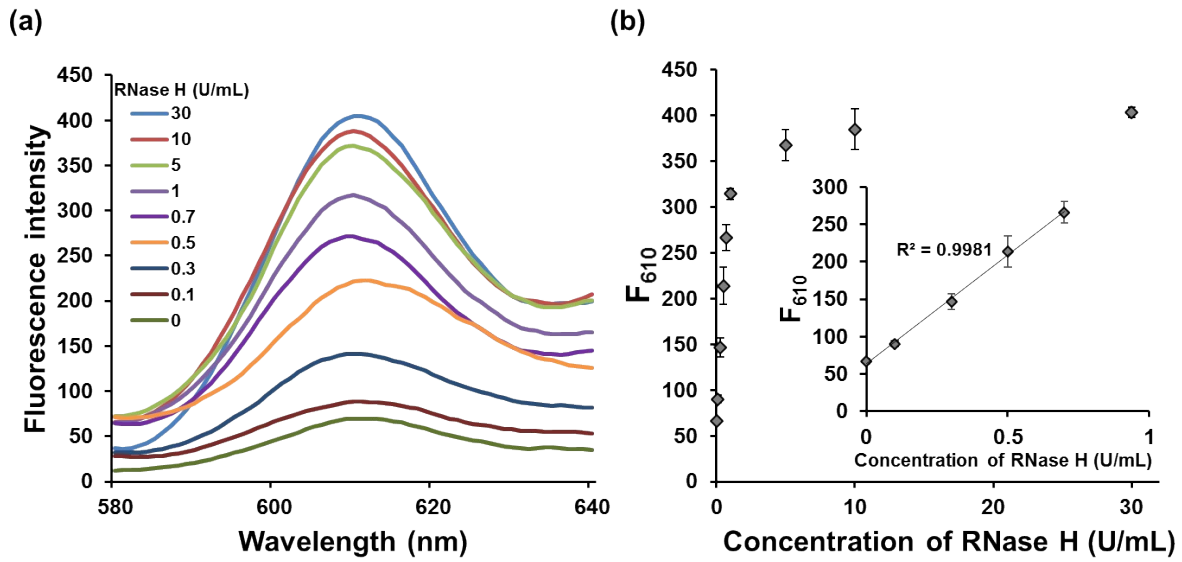


Fig. 3 (Enlarged version) Sensitivity of the RNase H activity assay. (a) Fluorescence emission spectra and (b) Fluorescence intensities at 610 nm from NMM in the presence of RNase H at varying concentrations. Inset in (b): linear relationship between F_{610} and RNase H concentration (0-0.7 U/mL).

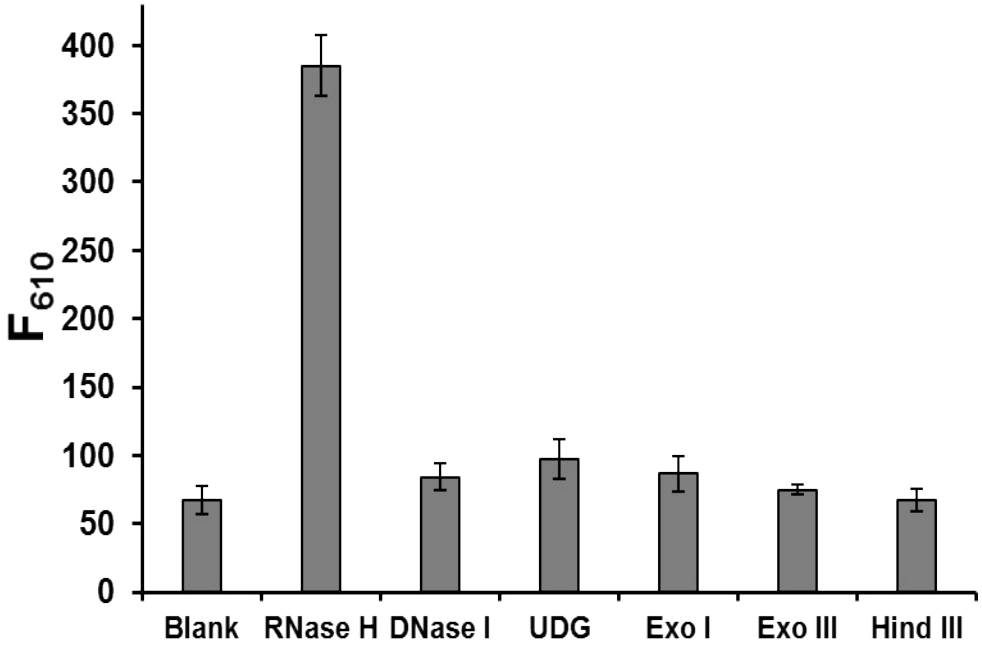


Fig. 4 (Enlarged version) Specificity of the RNase H activity assay. Fluorescence intensities at 610 nm in the presence of RNase H (10 U/mL) and other enzymes (50 U/mL).

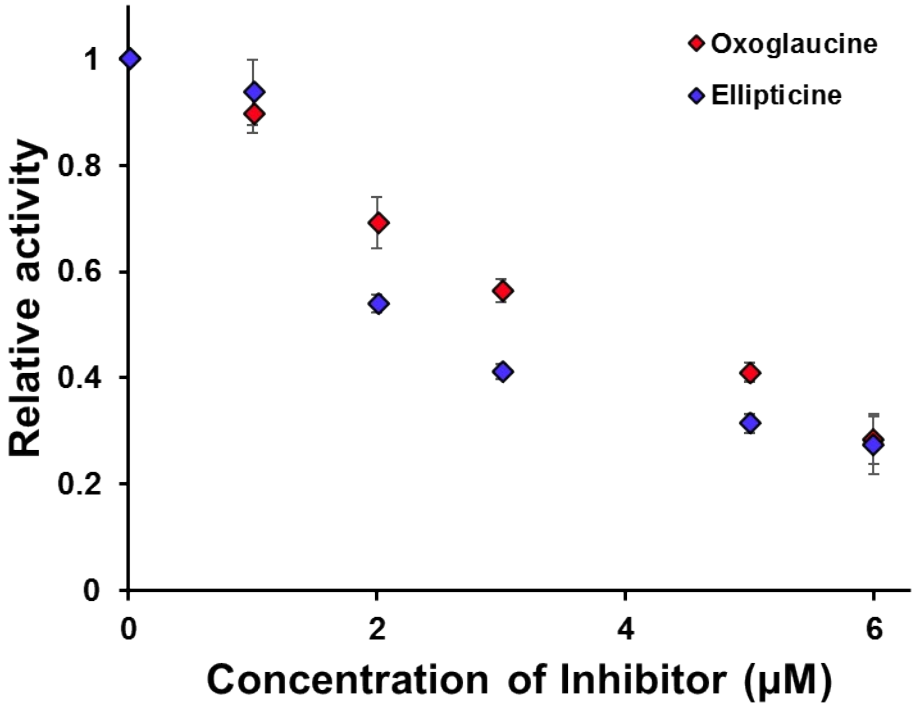


Fig. 5 (Enlarged version) Screening assay for the RNase H inhibitors. Relative activities in the presence of varying concentrations of oxoglaucine (red) and ellipticine (blue), respectively. The relative activity is defined as the ratio of the fluorescence intensity at 610 nm from NMM in the presence of varying concentrations of inhibitor to that in the absence of inhibitor. The final concentration of RNase H is 10 U/mL.

References

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