Supporting Information

Biotinylation and Isolation of RNA G-quadruplex Based on Its Peroxidase-mimicking Activity

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Experimental Methods

Materials and Equipments. All the oligonucleotides were synthesized and purified by Takara Biotechnology (Dalian, China). Reagents and starting materials for chemical syntheses were obtained from commercial suppliers (Sigma-Aldrich or Alfa Aesar) unless otherwise indicated. Biotin tyramide amd biotin-SS-tyramide were purchased from Iris. Biotin tyramide were dissolved in dimethyl sulfoxide as a 100 mM stock. Hemin or Fe(III)-heme (Frontier Scientifc) stock solutions were prepared fresh in dimethyl sulfoxide to 50 μM. H_2O_2 working solution was diluted from a 17.4 M stock to give 10 mM in nuclease-free water.

Biotinylation reaction for selectivity of biotinylation. The reactions were performed in a reaction buffer containing 40 mM HEPES pH 8.0, 150 mM potassium chloride, 1% dimethylformamide, 0.05% Triton X-100. The other components used in this reaction with a final concentration were 2 μM RNA stock solutions, 2 ug random oligo DNA (random oligo DNA was mixture of several DNA oligos), 5 μM hemin, 25 μM biotin tyramide and 1 mM H_2O_2. The total volume was 20 μL. Firstly, the mixture of RNA and random oligo DNA was heated to 70 °C for 3 min, then cooled to room temperature. Then hemin was added and incubated at room temperature for 10 min. After addition of biotin tyramide, the samples were rested for 10 min prior to initiating the biotinylation reaction at 22 °C for 5 min by the addition of H_2O_2. Reactions were quenched by quencher buffer (100 mM NaN_3, 100 mM sodium ascorbate). The unreacted biotin tyramide and other chemicals were removed by oligo Clean & Concentrator column.

Biotinylation reaction for optimizing the ideal conditons. RNA BCL2 was used for these reactions. The operations were same as biotinylation reaction for selectivity of biotinylation except for the changes in the conditions.

Biotinylation reaction for evaluating the self-biotinylation. The reactions were performed in a reaction buffer containing 40 mM HEPES pH 8.0, 150 mM potassium chloride, 1% dimethylformamide, 0.05% Triton X-100. The other components used in this reaction with a final concentration were 2 μM RNA stock solutions, 1 μg random oligo DNA (random oligo DNA was mixture of several DNA oligos), 5 μM hemin, 100 μM biotin tyramide and 1 mM H_2O_2. The total volume was 10 μL. Firstly, the mixture of RNA and random oligo DNA was heated to 70 °C for 3 min, then cooled to room temperature. Then hemin was added and incubated at room temperature for 10 min. After addition of biotin tyramide, the samples were rested for 10 min prior to initiating the biotinylation reaction at 22 °C for 5 min by the addition of H_2O_2. Reactions were quenched by adding quencher buffer. After desalting, 1μL of RNase A/T1(Thermo Scientific) was added to 10 μL sample to eliminate the RNA at 37 °C for 30 min. The unreacted biotin tyramide and degraded RNAs were removed by oligo Clean & Concentrator column.

Dot-blot assay. 50 ng of each sample was spotted on an Amersham Hybond-N+ membrane (GE Healthcare). After the membrane being dried, crosslinked the oligonucleotide to the membrane by photo-linking with UV light (254 nm, 5 min twice) and then washed the membrane with 1×TBST twice. The membrane was blocked with 5% BSA (Biosharp, China) at 37 °C for 1 h or room temperature for 2 h, followed by being washed with 1×TBST twice. After being incubated with

streptavidin-HRP (1:2000) (Thermo Scientific) at 37 °C for 1 h, the membrane was washed with 1×TBST four times. Finally, the results were demonstrated by enhanced chemiluminescence (SuperSignal™ West Pico Chemiluminescent Substrate, Cat: 34077, Thermo Scientific) using Molecular Imager® ChemiDocTM XRS+ Imaging System (Bio-Rad).

In vitro transcription. In vitro transcribed (IVT) RNAs were obtained using the RNA template under the guidance of the manufacturer's protocol of T7 RNA Polymerase (Thermo Scientific).

Biotinylation reaction for the transcribed RNAs. The reactions were performed in a reaction buffer containing 40 mM HEPES pH 8.0, 150 mM potassium chloride, 1% dimethylformamide, 0.05% Triton X-100. The other components used in this reaction with a final concentration were 5 μM hemin, 100 μM biotin tyramide and 1 mM H_2O_2. The total volume was 20 μL. Firstly, 10 μg the mixture of 1×G4 RNA and background RNA (1 μg 1×G4 RNA and 9 μg background RNA) was heated to 70 °C for 3 min, then cooled to room temperature. After addition of biotin-SS-tyramide, the samples were rested for 10 min prior to initiating the biotinylation reaction at 22 °C for 5 min by the addition of H_2O_2. The total volume was 50 μL of each sample. The unreacted biotin-SS-tyramide and other chemicals were removed by RNA Clean & Concentrator column.

Isolation of biotinylated RNA G4s. Biotin binding buffer: 100 mM Tris-HCl pH 7.0, 10 mM EDTA, 1M NaCl. Biotin wash buffer: 10 mM Tris-HCl pH 7.0, 1 mM EDTA, 4 M NaCl, 0.2% Tween. 10 μL of Dynabeads® MyOne™ Streptavidin C1 (Thermo Scientific) was washed twice with 500 uL of Biotin Binding buffer. The beads were resuspended with binding buffer and 1 μL of RiboLock RNase Inhibitor (Thermo Scientific) was added to the beads. Stored the beads on ice until use. After addition of biotinylated produdct, the reaction mixture was incubated the reaction mixture at room temperature for 45 min. The beads were then collected on a magnetic plate. The beads were then resuspended and washed five times with Biotin wash buffer. Then the beads were washed twice with RNase-free water. To cleave the disulfide bond in biotin linker, 50 μL of 100 mM DTT solution was added into the beads. Finally, placed the sample on magnet rack and kept the supernatant which contain rG4 sequences. The rG4 sequences were purified by RNA Clean & Concentrator column and eluted with same volume of nuclease-free water.

Reverse transcription with specific primer. After adding 0.5 μL of enriched RNAs and 1 μL of 10 μM 1xG4 PCR R primer to 4.3 μL nuclease free water, the samples were denatured in 65°C for 5 min and then placed in ice. Next 2 μL of 5× reverse transcription buffer, 0.2 μL of RiboLock RNase Inhibitor, 2 μL of 10 mM dNTPs and 0.5 μL of RevertAid Reverse Transcriptase (Thermo Scientific) were added to the denatured sample to get the total volume of 10 μL, then incubating the reaction mixture at 45 °C for 1 h, the RevertAid Reverse Transcriptase was deactivated in 70 °C for 10 min.

qPCR analysis of enriched RNA G4s. qPCR was performed using a CFX-96 Real-Time System (Bio-Rad, USA). The mixture contained 0.1 μL template of the DNA from the reverse transcription, 1 μL of forward primer (10 μM), 1 μL of reverse primer (10 μM), 10 μL of 2 × HieffTM PCR SYBR® GreenMaster Mix and 7.9 μL ddH_2O to give a final volume of 20 μL. The

mixture was subject to qPCR according to the following thermal cycle: 95 °C for 5 min, 40 cycles of (95 °C for 10 s, 60 °C for 30 s, 72 °C for 20 s).

PCR analysis of enriched RNA G4s. PCR was performed using DreamTaq PCR Master Mix (2X) (Thermo Scientific). The mixture contained 0.1 μL template of the DNA from the reverse transcription, 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM), 10 μL of 2 × DreamTaq PCR Master Mix and 7.9 μL ddH_2O to give a final volume of 20 μL. The mixture was subject to PCR according to the following thermal cycle: 95 °C for 3 min, 15 cycles of (95 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s), 72 °C for 5 min. The primers used for PCR were the same as primers for qPCR.

Sanger sequencing analysis. PCR product obtained from PCR analysis of RNA G4s. PCR product was purified by DNA Clean & Concentrator TM-5 kit. The PCR fragments were inserted into plasmid T-vectors (pClone007 Simple Vector Kit or Blunt Cloning Kit) according to the manufactuer's instructions for Sanger sequencing.

mRNA isolation. Extracted total RNA from HeLa cells using TRIzol according to the manufacturer's instructions (Invitrogen). Then, mRNA was isolated from total RNA using Oligo $(dT)_{25}$ Dynabeads(NEB) under the manuals.

Examination of G-quadruplex in transcriptome. The operating steps were the same as operations for transcribed RNA, expect for the addition of 500 nM PDS and extending the input mRNA to 10 μg and extending the volume to 50 μL during the biotinylation reaction.

Table S1. RNA oligonucleotides used in this study.

Name	Sequence (5’ to 3’)
NC	UUGUACUACACAAAAGUACUG
R-00	GGGUAGGGCGGGUUGGG
R-11	GGGUA GGGCGGGUUGGGA
BCL2	AGGGGGCCGUGGGGUGGGAGCUGGGG
NRAS	AGGGAGGGGCGGGUCUGGG
TERRA	UUAGGGUUAGGGUUAGGGUUAGGG
TERRA-m1	UUAGGGUUACGGUUAGGGUUAGGG
TERRA-m2	UUAGGGUUAGCGUUAGGGUUAGGG
TERRA-m3	UUAGGGUUAGGCUUAGGGUUAGGG
TERRA-mut	UUACCGUUACCGUUACCGUUACCG

Table S2. The sequences of primers or template used in this study.

Name	Sequence (5’ to 3’)
d(TERRA)	TTAGGGTTAGGGTTAGGGTTAGGG
1×G4 PCR F primer	GAGACCCAAGCTTGGTACCG
1×G4 PCR R primer	TTATGTTTTTGGCGTCTTCC
T7 promoter	TAATACGACTCACTATAGG
1×G4 DNA template	TATGTTTTTGGCGTCTTCCATGGTGGCTCTCGAGAGTAACCCGCCCCTACCCGT CCCTAACGTTACTAGTGGATCCGAGCTCGGTACCAAGCTTGGGTCTCCCTATA GTGAGTCGTATTA
background DNA template	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTT TAACTTGCTATTTCTAGCTCTAAAACATGCCGAGGGCTCTCCGCTCCCCCTATA GTGAGTCGTATTA
Spinach DNA template	GACGCGACCAGTTACGGAGCTCACACTCTACTCAACAGTGCGTGTTTCGCACT GGACCCGTCCTTCACCATTTCGGTCGCGTCCCCTATAGTGAGTCGTATTA
random oligo DNA	1.GACGCAACGGATCGTAGTGTGGAGATGCTCAAGATACGATCCGTTGCGTCTA TA 2. CCCAACGGATCATTAGAGGAAGATACGTACAATGATCCGTTGGGTATAT 3.GACGCAACGGATCCCAGGTGTGCAGATACGAACTGGGATCCGTTGCGTCTAT A 4. TGGGTGTTCGTATCTTG AGTCCAGGTGTGCA

Transcribed RNA from DNA template in Table S2

Spinach RNA

GGACGCGACCGAAAUGGUGAAGGACGGGUCCAGUGCGAAACACGCACUG

UUGAGUAGAGUGUGAGCUCCGUAACUGGUCGCGUC

1x G4 RNA:

GAGACCCAAGCUUGGUACCGAGCUCGGAUCCACUAGUAACGUUAGGGACGGGUAG GGGCGGGUUACUCUCGAGAGCCACCAUGGAAGACGCCAAAAACAUA 101 nt

Background RNA:

GGGAGCGGAGAGCCCUCGGCAUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAG GCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUU 102 nt

Biotinylation reaction for evaluating the self-biotinylation

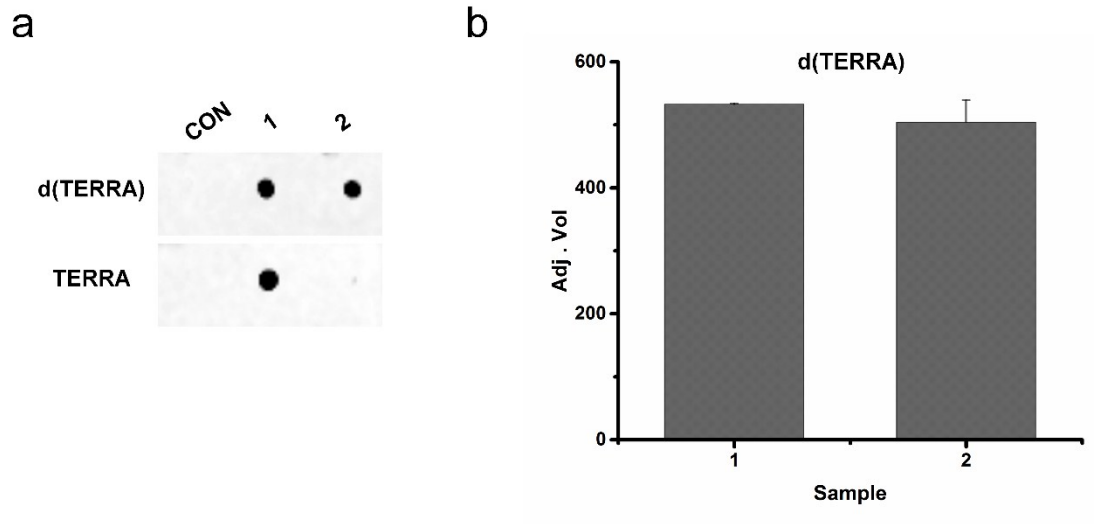

Figure S1. Self-biotinylation of RNA G4s. a) dot blot analysis of d(TERRA) and TERRA. CON, the sample underwent biotinylation reaction with the random oligo DNA; sample 1, the sample underwent biotinylation reaction under the optimized conditions; sample 2, RNA was digested by RNase A/T1 after the biotinylation reaction. b) The histogram exhibited the corresponding intensity of the dot blot analysis of d(TERRA). Adj . Vol indicated the adjust volume of the biotin signal.

Effect of mutated RNA G quadruplex on the biotinylation reaction

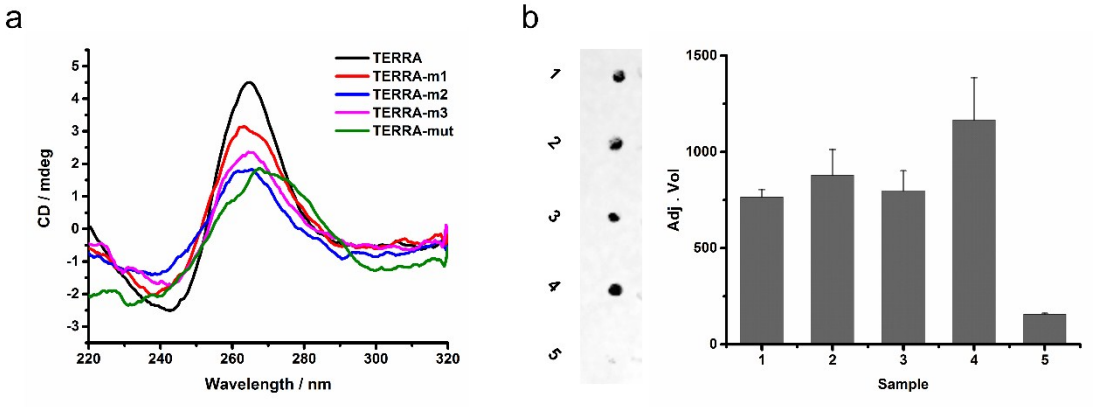

Figure S2. Effect of mutated RNA G quadruplex on the biotinylation reaction Self-biotinylation of RNA G4s. a) CD spectrums of TERRA and its mutants, buffer for CD: 10 mM Tris-HCl (pH 7.5), 150 mM KCl. TERRA-m1, TERRA-m2 and TERRA-m3 were TERRA mutated only one G in different layer, TERRA-mut indicated that TERRA mutated two Gs in each layer. b) Dot blot analysis and histogram of the biotinylated mutants. Sample 1 to 5 contained TERRA, TERRA-m1, TERRA-m2, TERRA-m3, TERRA-mut, respectively. Adj . Vol indicated the adjust volume of the biotin signal.

Biotinylation reaction of Spinach RNA

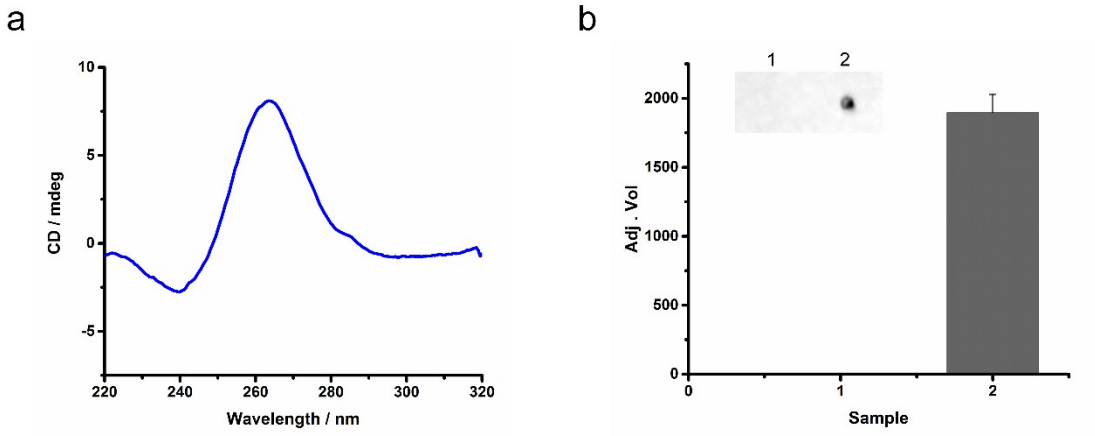

Figure S3. Biotinylation reaction of Spinach RNA. a) CD spectrum of Spinach RNA, buffer for CD: 10 mM Tris-HCl (pH 7.5), 150 mM KCl. b) Dot blot analysis and histogram of Spinach RNA, sample 1: Spinach RNA underwent the biotinylation reaction in absence of hemin, sample 2: Spinach RNA underwent the biotinylation reaction in presence of hemin. Adj . Vol indicated the adjust volume of the biotin signal.

Sanger sequencing of the PCR product

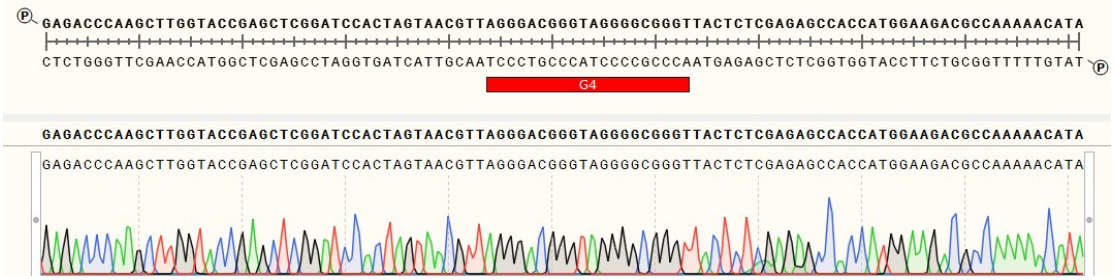

Figure S4. PCR product obtained from PCR analysis of enriched RNA G4s.

Examination of G-quadruplex in transcriptome

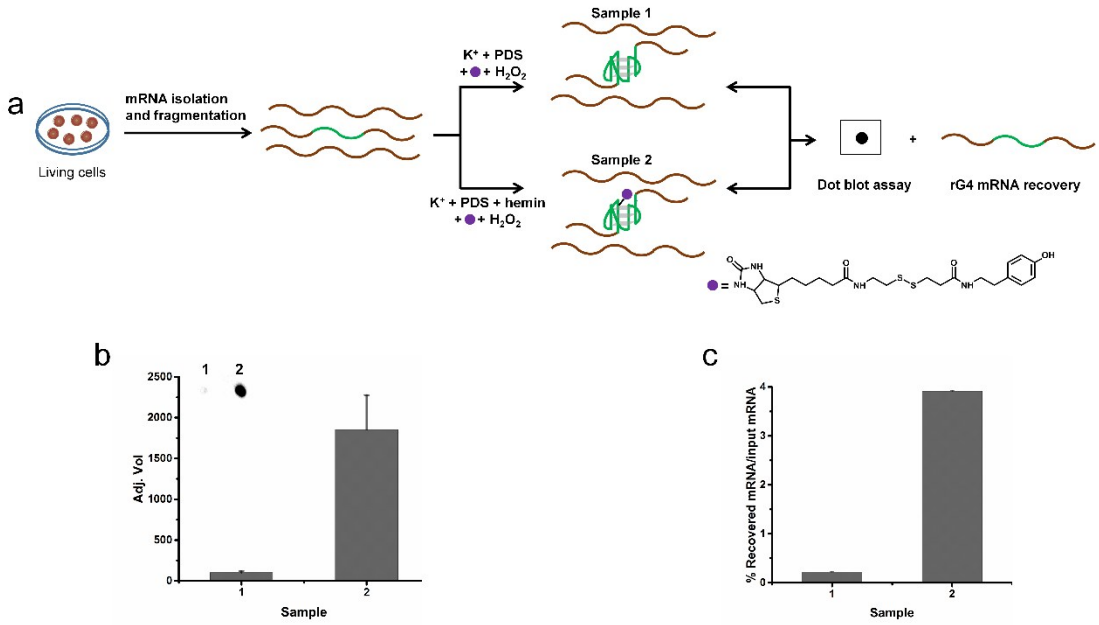

Figure S5. Examination of G-quadruplex in transcriptome. a) Scheme of examinating the existence of G-quadruplex in transcriptome. Sample 1: the biotinylation reaction was conducted with mRNA in absence of hemin; sample 2: the biotinylation reaction was conducted with mRNA under the optimized conditions. Reaction conditions: 5 μM hemin, 500 nM PDS. b) Dot blot assay, 100 ng product was loaded on the membrane. c) The comparison of pull-down yield. The yield was calculated by the ratio of recovered mRNA to input mRNA after being pulled down. Adj . Vol indicated the adjust volume of the biotin signal.