Supplementary Material for

Targeting nucleic acids with a G-triplex-to-G-quadruplex transformation and stabilization by a peptide-PNA G-tract conjugate

EXPERIMENTAL SECTION

Distribution of G3- and G4-forming sequences in the human genome

Genome sequence in fasta format of the human genome version hg38 was downloaded from the UCSC Genome Browser (https://genome.ucsc.edu/). The occurrence of G3- and G4-forming sequences was searched with a perl script as described (1) using a regular expression G{3,}(.{1,7}?G{3,}){0,} that represents a sequence motif carrying one or more guanine tracts (G-tracts), each with at least three guanine residues. The found motifs were then classified according to their number of G-tracts. Motifs with exactly three G-tracts were designated as G3 motifs and those with four or more G-tracts were designated as G4 motifs. The bed files of the G3 and G4 motifs were converted to bedGraph format using the Genomecov tool from the Bedtools software and then further converted to bigwig format using the bedGraphToBigWig tool from https://genome.ucsc.edu/. The occurrence frequency of the G3 and G4 motifs crossing transcription start sites (TSS) were obtained using the computeMatrix and plotProfile tools from the Deeptools software with the bed file of the Refseq genes downloaded from (https://genome.ucsc.edu/).

Oligonucleotides, peptides, linear DNA, and plasmids

5' fluorescently labeled oligonucleotides were purchased from Takara Biotechnology. Other oligonucleotides were purchased from Sangon Biotechnology (Beijing, China). RHAU-3G, RHAU23, and 3G PNA were purchased from SBS Genetech (Beijing, China). Synthesized peptides were purified by HPLC (Figure S1) followed by characterization on a MALDI-TOF/TOF Ultraflextreme mass spectrometer (Brucker, Germany) in positive ion reflectron mode (Figure S2).

DMS footprinting

Oligonucleotides were dissolved at 50 nM in 50 mM Tris-HCl buffer (pH 7.4) containing 150 mM KCl, heated at 95 °C for 10 min, and then cooled down at room temperature. They were then incubated in a 200 μL volume with the indicated ligand on ice for 1 hour before being subjected to DMS footprinting as previously described (2).

Electrophoretic mobility shift assay (EMSA)

Oligonucleotide (2.5 nM) was dissolved in a buffer containing 20 mM Tris-HCl (pH 7.4), 150 mM KCl, 50 nM poly-T, heated at 95 °C for 10 min, and then cooled down at room temperature. The sample was then incubated with the indicated compound and 0.2 mg/mL BSA on ice for 1 hour, resolved on a 16% non-denaturing polyacrylamide gel containing 150 mM KCl at 4 °C for 3 hours in a 1× TBE buffer containing 150 mM KCl. DNA was visualized by the FAM dye covalently labeled at the 5' end of the oligonucleotide on a Typhoon Trio Imager (GE Healthcare) and digitized using the Image Quant 5.2 software.

DNA tracking activity assay

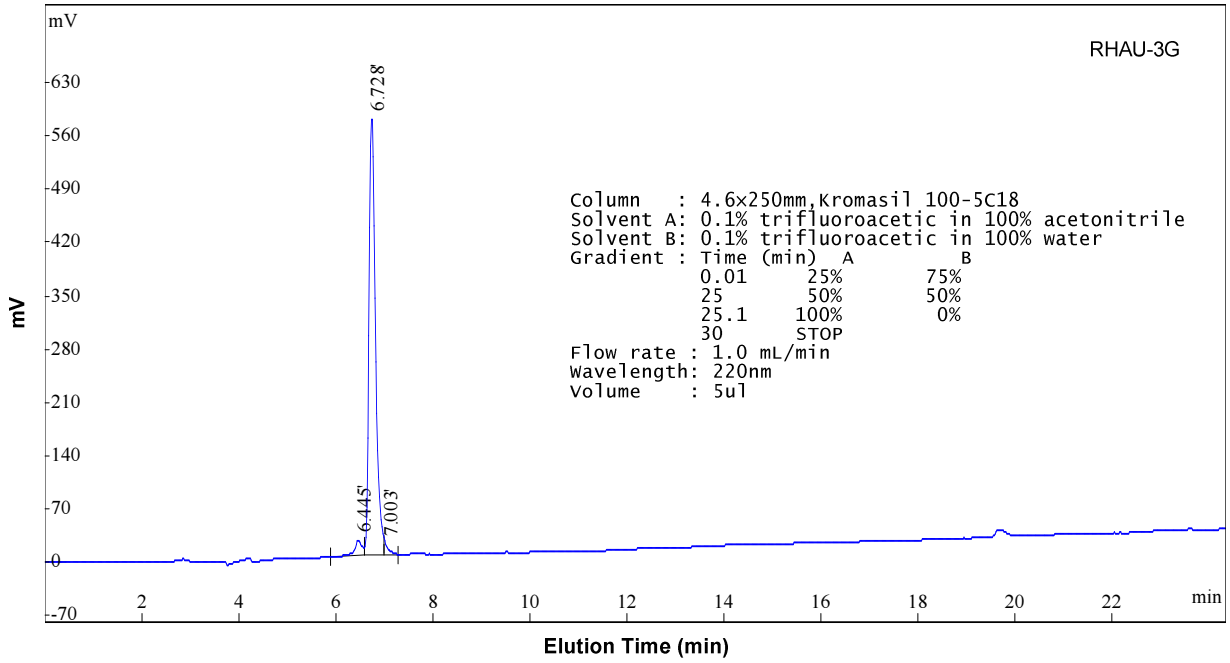
Expression and purification of $BLM^{642-1296}$ were carried out as previously described (3). DNA substrate was prepared by annealing 1 μM G3 single-stranded DNA (ssDNA) with 1 μM 5'-TAMRA-labeled probe (5'- TAMRA-CTCTGCTCAACGGAAC-3') in a buffer of 20 mM Tris-HCl (pH 7.4), 50 mM LiCl by heating at 95 °C for 5 min followed by cooling down to 20 °C at a rate of 0.03 °C/s. It was then diluted to a final concentration of 5 nM into 20 mM Tris (PH7.4) buffer containing 150 mM KCl, 50 nM poly-T, 0.2 mg/ml BSA, and the indicated ligand and incubated at 25 °C for 30 min. Unwinding was carried out on a Spex Fluorolog-3 spectrofluorometer (HORIBA Jobin Yvon, France) at 25 °C in a 2mL volume by addition of 2 mM $MgCl_2$, 0.5 mM ATP, 50 nM BLM helicase under constant stirring. Change in fluorescence was monitored in real-time with excitation wavelength set at 555 nm and emission at 575 nm.

Table S1. Oligonucleotides used in this study (5'—3')

Kd determination	
TEL-G3	6-FAM-AGGGTTAGGGTTAGGGT
TEL-G3M	6-FAM-AGAGTTAGAGTTAGGGT
TEL-G4	6-FAM-AGGGTTAGGGTTAGGGTTAGGGT
TERT-G3	6-FAM-AGGGGGAGCGGGGCGTGGGGGT
TERT-G3M	6-FAM-AGTGAGAGCGGGGCGTGAGTGT
TERT-G4	6-FAM-AGGGGATAGGGGGAGCGGGGCGTGGGGGT
HIV-G3	6-FAM-TGGGACGCAGGGGGTGGGA
HIV-G3M	6-FAM-TGAGACGCAGTGAGTGGGA
HIV-G4	6-FAM-TGGGAGGGACGCAGGGGGTGGGA

DNA tracking activity assay	
TEL-G3	GTTCCGTTGAGCAGAGTTAGGGTTAGGGTTAGGGTTATTGTTAG
HIV-G3	GTTCCGTTGAGCAGAGTTAGGGACGCAGGGGGTGGGTTATTGTTAG
TERT-G3	GTTCCGTTGAGCAGAGTTAGGGGGAGCGGGGCGTGGGGGTTATTGTTAG
Random	GTTCCGTTGAGCAGAGTTAGCATGCGAGCCTTATTGTTAG
TEl-G4	GTTCCGTTGAGCAGAGTTAGGGTTAGGGTTAGGGTTAGGGTTATTGTTAG
HIV-G4	GTTCCGTTGAGCAGAGTTAGGGAGGGACGCAGGGGGTGGGTTATTGTTAG
TERT-G4	GTTCCGTTGAGCAGAGTTAGGGGATAGGGGGAGCGGGGCGTGGGGGTTATTGTTAG
Blue region hybridized with TAMRA-CAAGGCAACTCGTCTC	

Figure S1. HPLC characterization of synthesized RHAU23, RHAU-3G.



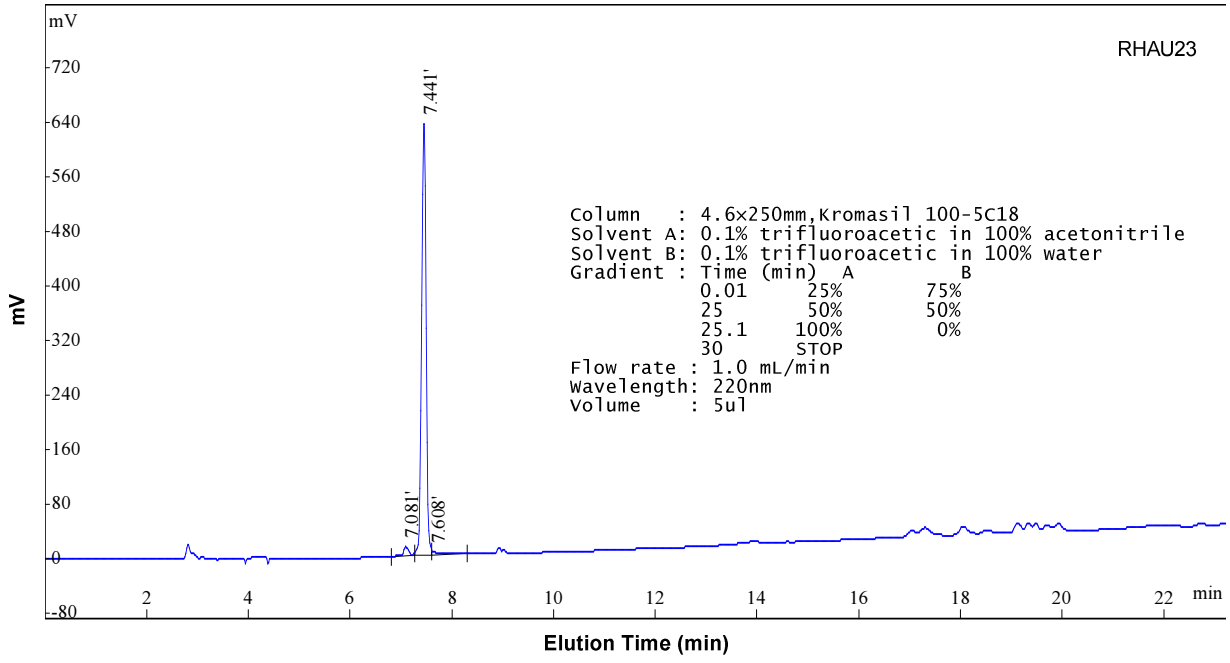
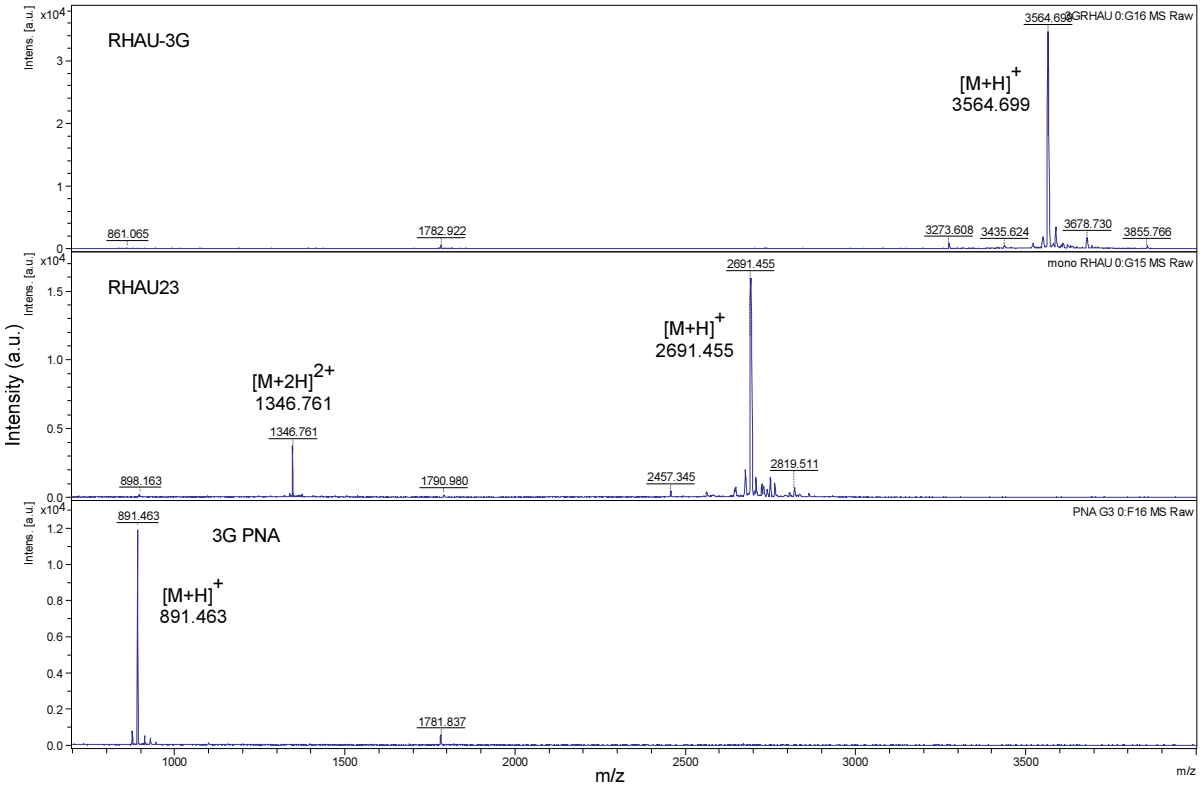


Figure S2. MALDI-TOF mass spectra of RHAU23, RHAU-3G, and 3G PNA.



References

1. Xiao, S., Zhang, J.Y., Zheng, K.W., Hao, Y.H. and Tan, Z. (2013) Bioinformatic analysis reveals an evolutional selection for DNA:RNA hybrid G-quadruplex structures as putative transcription regulatory elements in warm-blooded animals. *Nucleic Acids Res.*, **41**, 10379-10390.
2. Li, X.M., Zheng, K.W., Zhang, J.Y., Liu, H.H., He, Y.D., Yuan, B.F., Hao, Y.H. and Tan, Z. (2015) Guanine-vacancy-bearing G-quadruplexes responsive to guanine derivatives. *Proc. Natl. Acad. Sci. USA*, **112**, 14581-14586.
3. Liu, J.Q., Chen, C.Y., Xue, Y., Hao, Y.H. and Tan, Z. (2010) G-quadruplex hinders translocation of BLM helicase on DNA: a real-time fluorescence spectroscopic unwinding study and comparison with duplex substrates. *J. Am. Chem. Soc.*, **132**, 10521-10527.