Supporting Information

Estimation of G-Quartet-Forming Guanines in Parallel-Type G-Quadruplexes by Optical Spectroscopy Measurements of Their Single-Nucleobase Substitution Sequences

Ryo Maruyama[a], Kurumi Makino[a], Toru Yoshitomi[a], Hiroharu Yui[b], Hitoshi Furusho[c], and Keitaro Yoshimoto[a, d]

[a] Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro, Tokyo 153-8902, Japan.

[b] Department of Chemistry, Faculty of Science, Tokyo University of Science, 1-3 Kagurazaka, Shinjuku, Tokyo 162-8601, Japan.

[c]Chemical General Division, Nissan Chemical Industries, Ltd., 2-10-2 Tsuboi-nishi, Funabashi, Chiba 274-8507, Japan.

[d]JST, PRESTO, The University of Tokyo, Komaba 3-8-1, Meguro-ku, Tokyo 153-8902, Japan.

* Corresponding author: Keitaro Yoshimoto, Ph.D., Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Komaba 3-8-1, Meguro-ku, Tokyo 153-8902, Japan

Phone and FAX: +81-3-5454-6580

E-mail: ckeitaro@mail.ecc.u-tokyo.ac.jp

EXPERIMENTAL

Sample preparation

The lyophilized oligonucleotides were purchased from Sigma–Aldrich (Japan) and resuspended in Milli-Q water at a concentration of 100 μM, which was used as a stock solution. The oligonucleotide sequences used in this study are shown in Table S1-3. Each oligonucleotides solution was prepared with a different buffer, as reported previously. T95-2T, Pu22-T12T13, and VEGF-Pu22 were prepared with DPBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.5 mM KH_2PO_4, pH 7.4), 10 mM potassium phosphate buffer containing 40 mM KCl (pH 7.2), and 25 mM potassium phosphate buffer containing 70 mM KCl (pH 7.0), respectively. Before use, all samples were heated to 90 °C for 2.5 min and allowed to cool to 4 °C slowly.

Circular dichroism (CD) measurements. CD spectra were measured using a J-1500 CD Spectrometer (JASCO Corporation, Japan) with a 1-cm path length quartz cuvette and constant flow of dry nitrogen. Scans were performed three times from 220 to 320 nm at 100 nm/min with a 1-s response time, 0.5-nm pitch, and 1-nm bandwidth. The CD spectrum of a blank sample containing buffer alone was measured in the same manner and subtracted from the collected data.

UV melting measurements. UV melting curves were collected using a TMSPC-8 system with a UV-2450 spectrophotometer (Shimadzu, Japan), and the absorbance was measured at 295 nm. The samples were transferred to a 1-cm path length quartz cuvette and then covered with a layer of liquid paraffin. Melting measurements were performed at a heating rate of 0.5 °C/min, and a constant flow

of dry nitrogen was used to prevent dew formation. T_m was estimated from the first derivative of the melting curves.

FIGURES

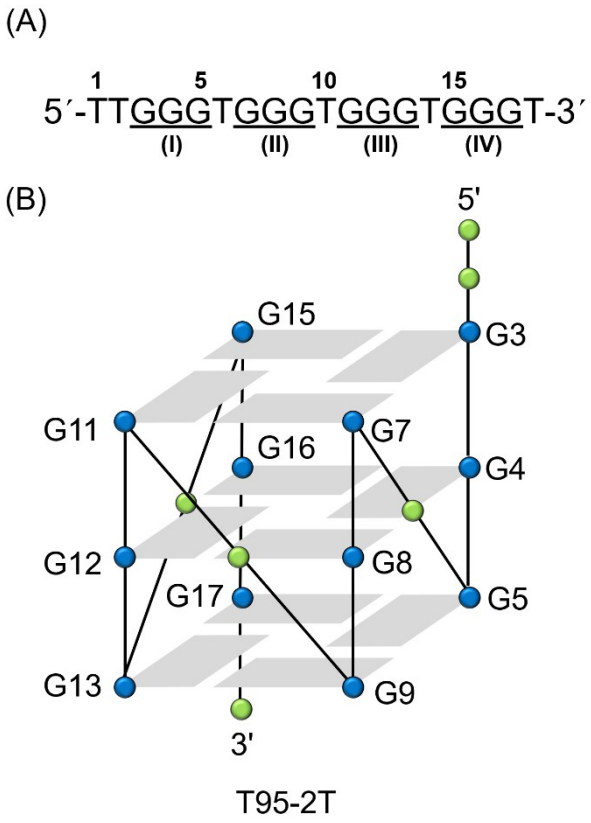

Fig. S1 (A) The T95-2T sequence. T95-2T is an 18-mer G-rich sequence. The four G-runs are underlined and numbered (I–IV). The numbers above the sequence indicate the base number. (B) Three-dimensional structure of parallel type T95-2T G-quadruplex, which was created based on PDB ID 2LK7 (color scheme: G-quartet-forming guanine (G), blue; thymine (T), green).

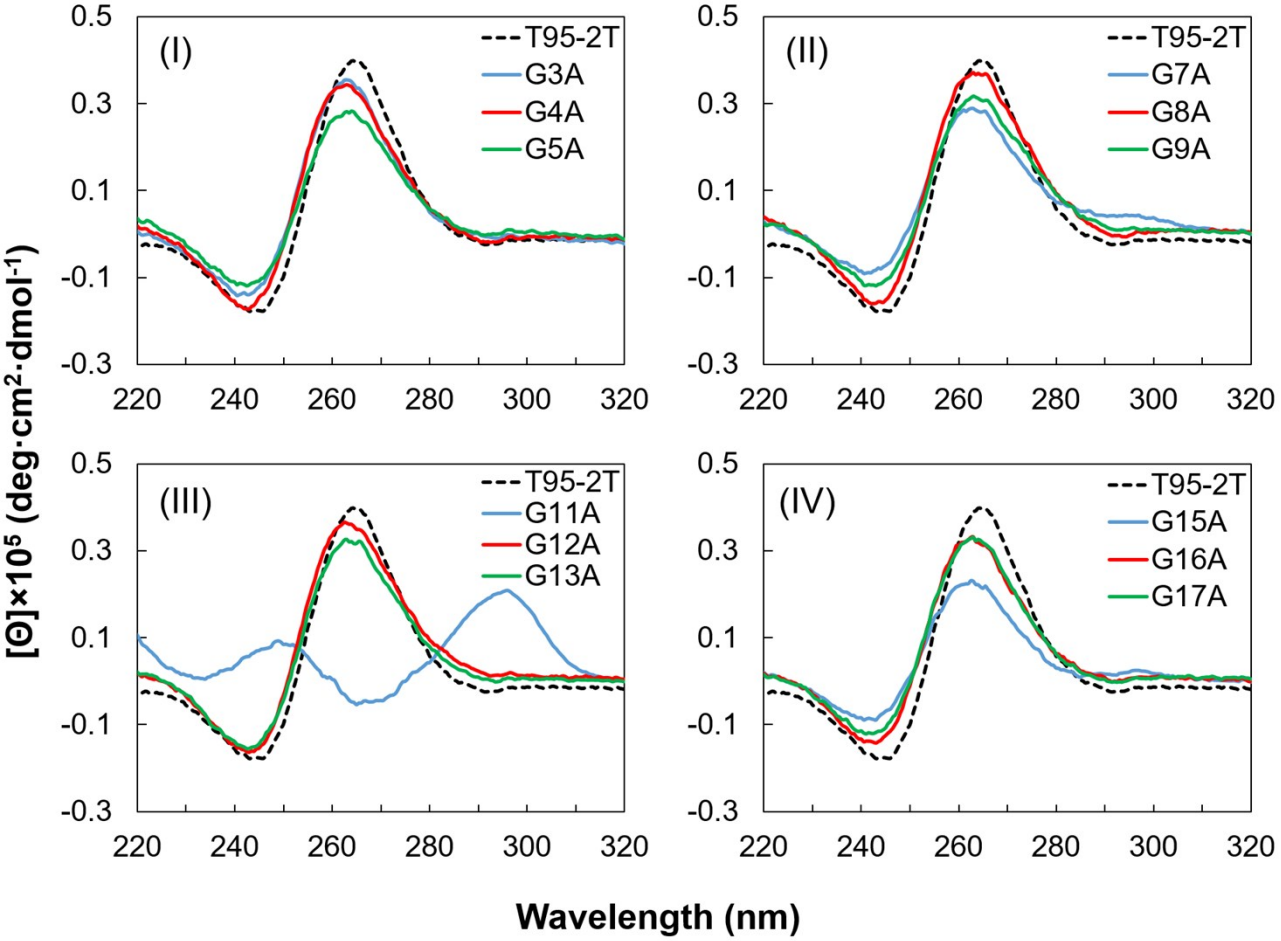

Fig. S2 CD spectra of the T95-2T sequence and its single-nucleobase G-to-adenine (A) substitution sequences measured in DPBS (pH 7.4). The concentration of the ssDNA sample was 4 μM.

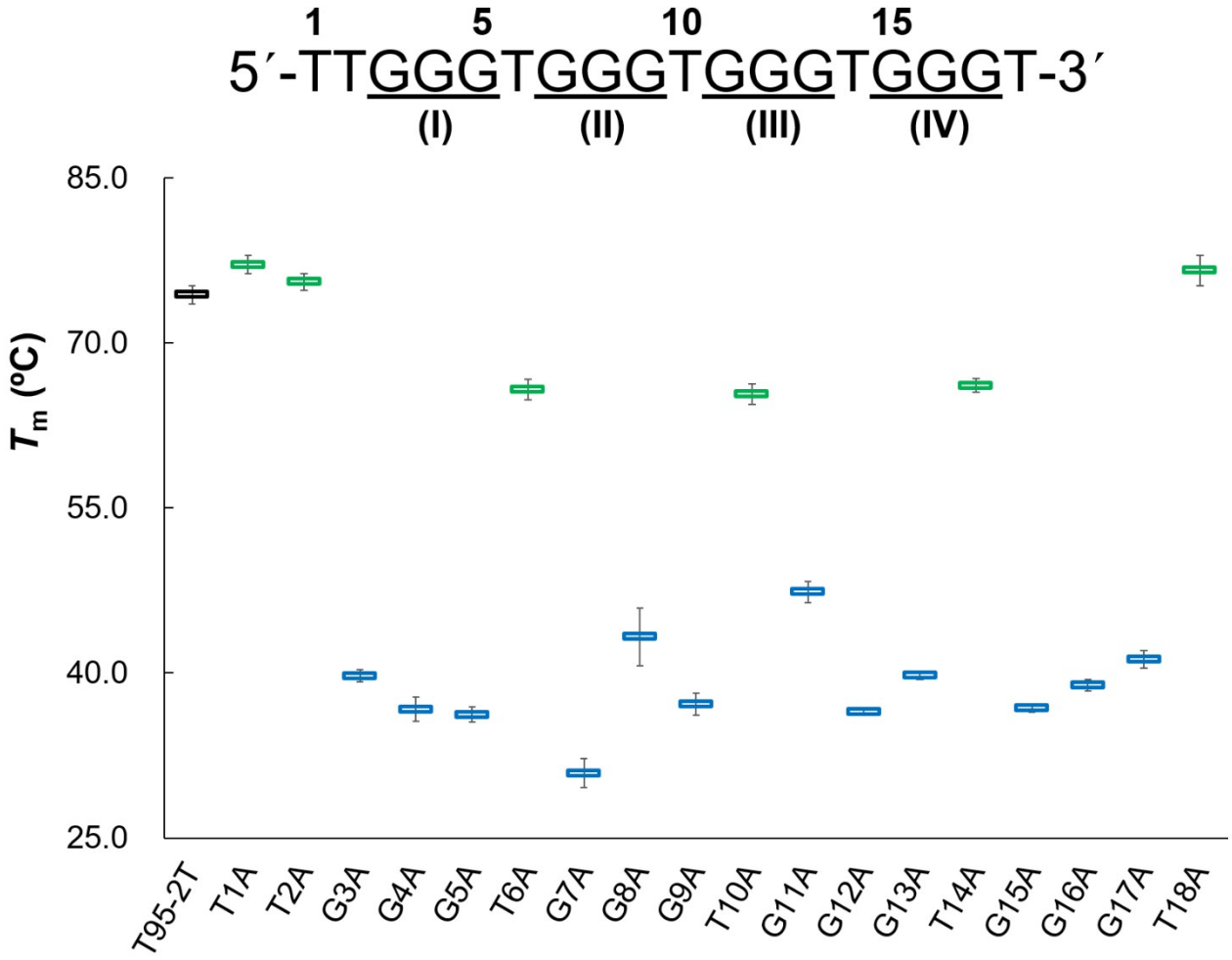

Fig. S3 T_m values of the T95-2T sequence and its single-nucleobase G-to-A substitution sequences. The T_m values of 4 µM of each sequence in DPBS (pH 7.4) were measured from the UV melting curves at 295 nm (mean values; n = 3; error bars, SD). T_m values of T95-2T without substitution are shown in black, where T_m is 74.4 ± 0.8 °C. The green points indicate the T_m values of the T-to-A substitutions. The blue points are the G-quartet-forming Gs in the T95-2T sequence. The four G-runs are underlined and numbered (I–IV). The number above the sequence indicates the base number.

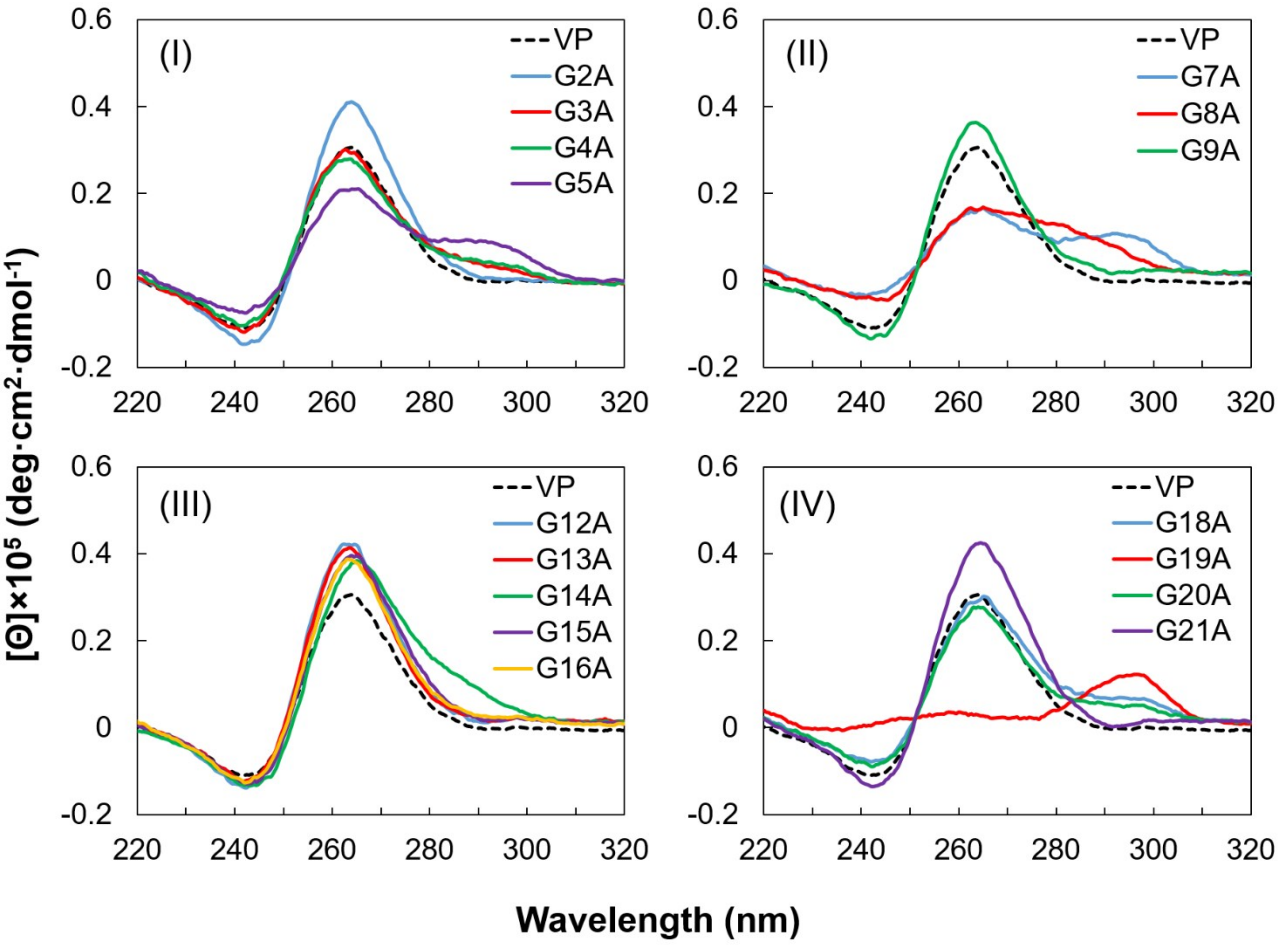

Fig. S4 CD spectra of the VEGF_Pu22 (VP) sequence and its single-nucleobase G-to-A substitution sequences. The 4-μM ssDNA samples were prepared in 25 mM K-phosphate buffer containing 70 mM KCl (pH 7.0).

TABLES

Table S1 VEGF_Pu22T12T13T and its single-nucleobase G-to-A substitution sequences.

Name	Sequence (5′ to 3′)
VEGF_Pu22T12T13 (VPT)	CGGGGCGGGCCTTGGGCGGGGT
VPT_G2A	CAGGGCGGGCCTTGGGCGGGGT
VPT_G3A	CGAGGCGGGCCTTGGGCGGGGT
VPT_G4A	CGGAGCGGGCCTTGGGCGGGGT
VPT_G5A	CGGGACGGGCCTTGGGCGGGGT
VPT_G7A	CGGGGCAGGCCTTGGGCGGGGT
VPT_G8A	CGGGGCGAGCCTTGGGCGGGGT
VPT_G9A	CGGGGCGGACCTTGGGCGGGGT
VPT_G14A	CGGGGCGGGCCTTAGGCGGGGT
VPT_G15A	CGGGGCGGGCCTTGAGCGGGGT
VPT_G16A	CGGGGCGGGCCTTGGACGGGGT
VPT_G18A	CGGGGCGGGCCTTGGGCAGGGT
VPT_G19A	CGGGGCGGGCCTTGGGCGAGGT
VPT_G20A	CGGGGCGGGCCTTGGGCGGAGT
VPT_G21A	CGGGGCGGGCCTTGGGCGGGAT

Table S2 T95-2T and its single-nucleobase adenine substitution sequences.

Name	Sequence (5′ to 3′)
T95-2T	TTGGGTGGGTGGGTGGGT
T95-2T_T1A	ATGGGTGGGTGGGTGGGT
T95-2T_T2A	TAGGGTGGGTGGGTGGGT
T95-2T_G3A	TTAGGTGGGTGGGTGGGT
T95-2T_G4A	TTGAGTGGGTGGGTGGGT
T95-2T_G5A	TTGGATGGGTGGGTGGGT
T95-2T_T6A	TTGGGAGGGTGGGTGGGT
T95-2T_G7A	TTGGGTAGGTGGGTGGGT
T95-2T_G8A	TTGGGTGAGTGGGTGGGT
T95-2T_G9A	TTGGGTGGATGGGTGGGT
T95-2T_T10A	TTGGGTGGGAGGGTGGGT
T95-2T_G11A	TTGGGTGGGTAGGTGGGT
T95-2T_G12A	TTGGGTGGGTGAGTGGGT
T95-2T_G13A	TTGGGTGGGTGGATGGGT
T95-2T_T14A	TTGGGTGGGTGGGAGGGT
T95-2T_G15A	TTGGGTGGGTGGGTAGGT
T95-2T_G16A	TTGGGTGGGTGGGTGAGT
T95-2T_G17A	TTGGGTGGGTGGGTGGAT
T95-2T_T18A	TTGGGTGGGTGGGTGGGA

Table S3 VEGF_Pu22 and its single-nucleobase G-to-A substitution sequences.

Name	Sequence (5′ to 3′)
VEGF_Pu22 (VP)	CGGGGCGGGCCGGGGGCGGGGT
VP_G2A	CAGGGCGGGCCGGGGGCGGGGT
VP_G3A	CGAGGCGGGCCGGGGGCGGGGT
VP_G4A	CGGAGCGGGCCGGGGGCGGGGT
VP_G5A	CGGGACGGGCCGGGGGCGGGGT
VP_G7A	CGGGGCAGGCCGGGGGCGGGGT
VP_G8A	CGGGGCGAGCCGGGGGCGGGGT
VP_G9A	CGGGGCGGACCGGGGGCGGGGT
VP_G12A	CGGGGCGGGCCAGGGGCGGGGT
VP_G13A	CGGGGCGGGCCGAGGGCGGGGT
VP_G14A	CGGGGCGGGCCGGAGGCGGGGT
VP_G15A	CGGGGCGGGCCGGGAGCGGGGT
VP_G16A	CGGGGCGGGCCGGGGACGGGGT
VP_G18A	CGGGGCGGGCCGGGGGCAGGGT
VP_G19A	CGGGGCGGGCCGGGGGCGAGGT
VP_G20A	CGGGGCGGGCCGGGGGCGGAGT
VP_G21A	CGGGGCGGGCCGGGGGCGGGAT