A "sugar-deficient" G-quadruplex: incorporation of *a*TNA in G4 structures

Jun Zhou,[#ab] Keiji Murayama,[#c] Samir Amrane,[ab] Frédéric Rosu,[d] Hiromu Kashida,[c] Anne Bourdoncle,[bd] Hiroyuki Asanuma*[c] and Jean-Louis Mergny*[ab]

*Corresponding authors. Graduate School of Engineering, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8603, Japan. E-mail address: asanuma@nubio.nagoya-u.ac.jp (H. Asanuma); 2 rue Robert Escarpit, IECB Laboratoire ARNA, F-33607, Pessac, France. E-mail address: jean-louis.mergny@inserm.fr (J.-L. Mergny).

Supplementary information

Molecular Modeling

The G-quadruplex core of TNA3 was generated using Hyperchem 8.0.10 software. A single $(TG_4T)_4$ was extracted from the pdb file (2O4F). Sodium was replaced by potassium, thymine and water molecules were removed and hydrogen atoms added to the structure. We replaced the deoxyribose of the third nucleotide by *a*TNA to create structure TNA3 (a). The initial geometry of *a*TNA was obtained after optimization using Gaussian[1] at M062x/6-31G(d,p) level and charge fitting of *a*TNA was obtained using RESP/HF 6-31G(d) on a nucleoside. We flipped the third G-tetrad to create the structure (b). Parm99 force field with the parmbcs0 parameters was used. The backbone of structure (a) and (b) are first relaxed and optimized prior to molecular dynamics simulations. The structures are then subjected to restrained MD simulation for 1ns using a force constant of 10 kJ mol^{-1} A^{-2} on the H-bonds of the G-tetrads.

Molecular dynamics simulations were carried out using Gromacs 4.5.5 software[2] with particule mesh Ewald summation[3] under periodic boundary conditions. A truncated octahedral box of water is used to solvated the structures. SPC/E model was used to describe water molecules. The water molecules were constrained using the Settle algorithm and Lincs was used for the other bonds to allow a time step of 2 fs.100 ns of MD simulations were performed at 298 K and 1 atm.

1 Gaussian 09, Revision B.01, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian, Inc., Wallingford CT, 2009.

2 B. Hess, C.Kutzner, D. van der Spoel, E.Lindahl, *J. Chem. Theory Comp.*, 2008, **4**, 435–447.

3T. Darden, D. York, L. Pedersen, J. Chem. Phys. 1993, **98**, 10089–10092

Figure S1

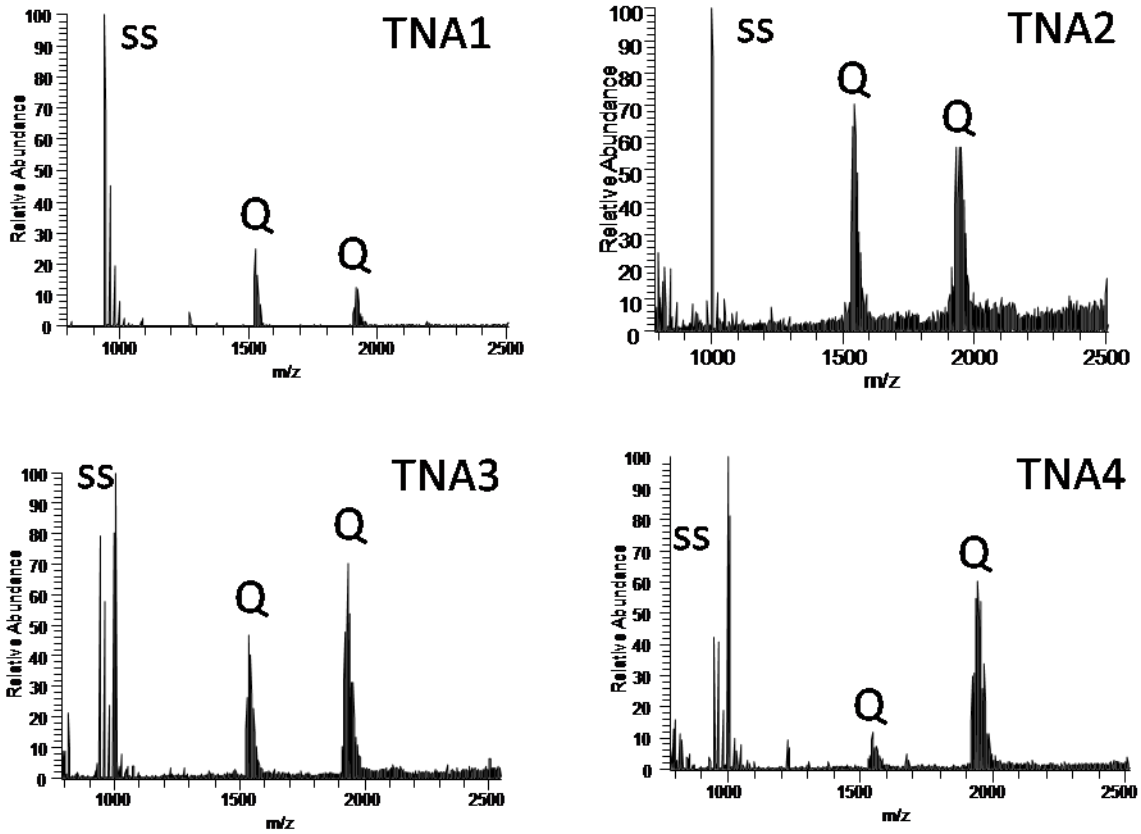

Fig. S1 ESI-MS spectra of the tetramolecular G-quadruplexes. The sample were prepared in 500 mM KCl at a strand concentration of 20 µM. Desalting against 150 mM NH_4OAc was performed prior to the mass analysis. The tetramolecular assembly is detected with 5 and 4 negative charges.

Figure S2

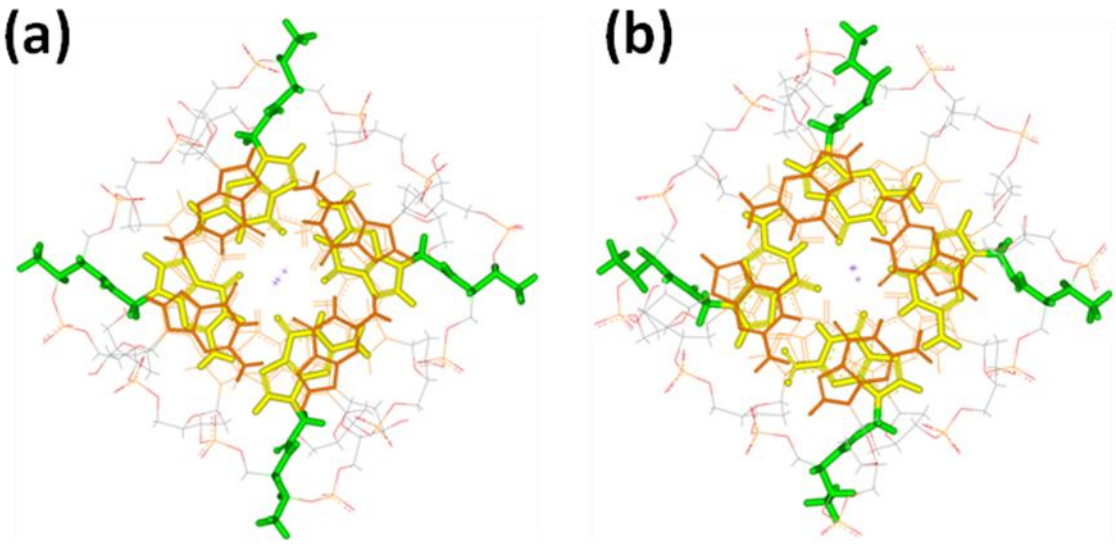

Fig. S2 Structure of TNA3 after 100 ns molecular dynamics modeling. (a) TNA3 with the G-tetrad having the same topology. (b) TNA3 with a flipped tetrad. The G-tetrads are represented in orange. In yellow, the third G-tetrad bearing the *a*TNA modification. *a*TNA backbone is shown in green.

Figure S3

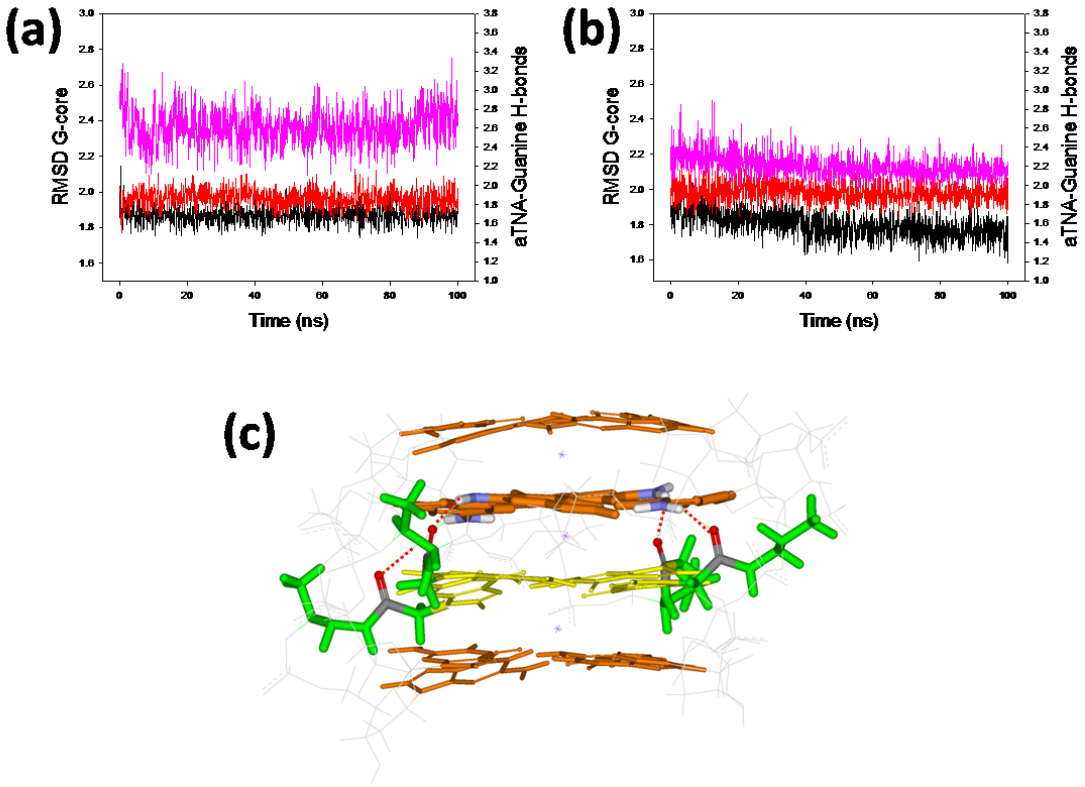

Fig. S3 (a) Left scale: Root mean square deviation of atoms (RMSD) as a function of time. Black line: G-core constituted of the 4 tetrads is taking into account. Red line: the third G-tetrad (attached to the *a*TNA backbone) only. Right scale: Cyan line: mean distances of the H-bonds between the C=O of *a*TNA and the amino group of the guanine. (a) TNA3 structure with all the G-tetrad parallel. (b) TNA3 structure with the third G-tetrad flipped. (c) Structure of TNA3 with the flipped tetrad (yellow). The other G-tetrads are in orange, *a*TNA is in green. The H-bonds between the C=O of aTNA and NH_2 of the guanine of the adjacent tetrad (5' side) are shown in red dots.

Figure S4

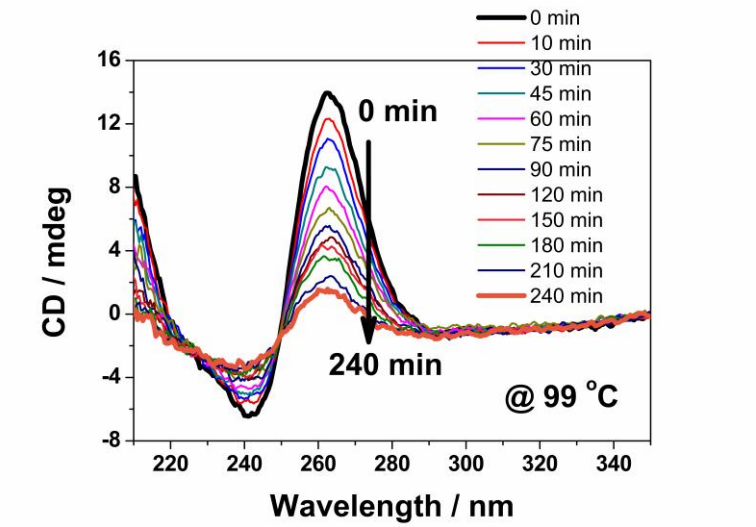

Fig. S4 CD spectra of 5 μM TG4T incubated at 99 ^{o}C in 10 mM cacodylate buffer (pH 7.0) containing 100 mM K^{+}. The spectrum are collected at different times (0 min- 240 min).

Figure S5

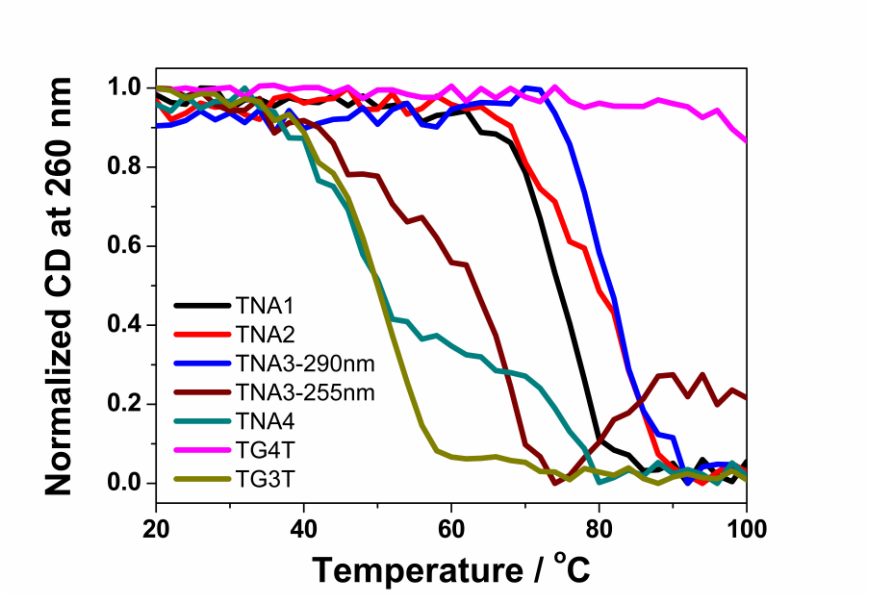

Fig. S5 CD melting profiles (The sample of TNA3 are measured at two wavelength 290 nm and 256 nm) of all the samples in 10 mM cacodylate buffer (pH 7.0) containing 100 mM K^+ at 260 nm.

Figure S6

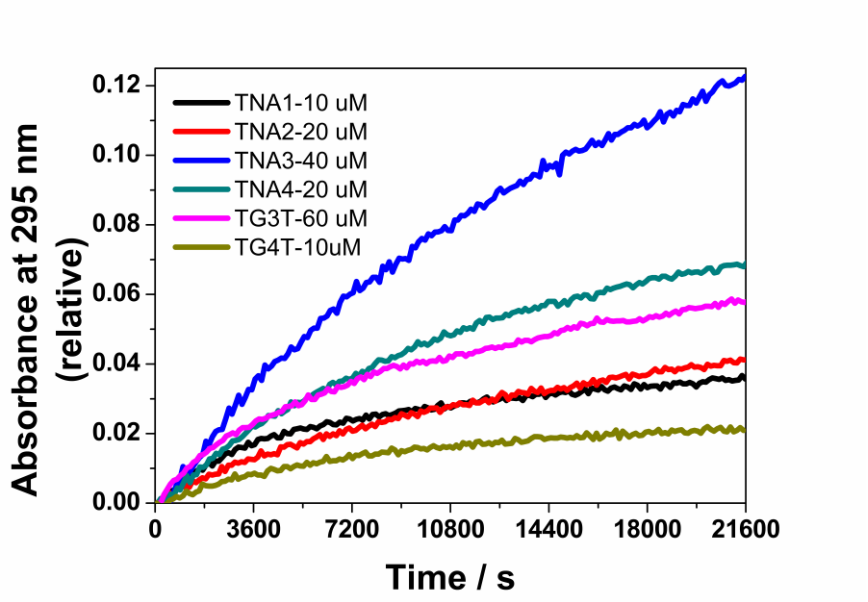

Fig. S6 Association kinetics of various samples (TNA1, TNA2, TNA3, TNA4, TG3T, and TG4T). The experiments are performed at 10 ^{o}C in the presence of 500 mM K^{+}. Oligonucleotide concentrations are shown in the figure and the absorbance is recorded at 295 nm.