Electronic Supplementary Information

Tetrad-binding ligands do not bind specifically to left-handed G-quadruplexes

Poulomi Das and Anh Tuân Phan*

Materials and Methods

DNA Sample Preparation

All the DNA samples were purchased from Integrated DNA Technologies. The samples were dissolved in respective buffer solution as listed in Table S1. DNA concentration of 100 μ M were used for all the titration experiments. They were either annealed or quenched depending on the sequence. Annealing was performed by heating the DNA samples at 95 °C for 5 minutes in a water bath and letting them cool down slowly to room temperature, whereas in quenching, the samples were heated at 95 °C for 5 minutes in a water bath and puickly cooled down to room temperature by placing it in ice water at 0 °C.

Ligand Preparation

PhenDC₃ was dissolved in DMSO with concentration of ~20 mM displaying no aggregation and Rhau23 dissolved in deionized water with concentration of ~8.3 mM were used for the experiments. Specific amount of the stock solutions were added to the NMR tube containing the DNA sample for the NMR titration experiments.

Nuclear Magnetic Resonance (NMR) Spectroscopy and NMR titration

All the NMR experiments were performed on 600 MHz Bruker AVANCE II spectrometer at 25 °C unless otherwise stated. DNA concentrations used was 100 μM. All spectra were processed and analyzed using TopSpin 4.0.6.

Circular Dichroism Spectroscopy

The circular dichroism (CD) spectra of all the DNA samples were measured with JASCO-815 spectropolarimeter. The respective DNA samples with excess of ligand (used for NMR) were diluted to a final concentration of ~4-5 μ M in their corresponding buffer solution. Subsequently, 500 μ l of the diluted DNA sample was transferred to a 1cm path-length cuvette and the CD spectrum was recorded at 25 °C with 10 accumulations. The data was baseline corrected from the signal contributed by the buffer. Each spectrum was further corrected to be zero at 320 nm wavelength.

Supplementary Table S1: List of DNA sample sequences, their respective buffer solutions in which they are dissolved and the method of treatment.

Sequence Name	Sequence (5'-3')	Buffer solution
Pu24T	TGAGGGTGGTGAGGGTGGGGAAGG	10 mM KPi, 35 mM KCl, pH = 7.0, 10%
		D ₂ O, 20 μM DSS
2xBlock2∆	GTGGTGGTGGTGTTGTGGTGGTGGTG	20 mM KPi, 70 mM KCl, pH = 7.0, 10%
		D ₂ O, 20 μM DSS
T95-2T	TTGGGTGGGTGGGTGGGT	10 mM KPi, 10 mM KCl, pH = 7.0, 10%
		D ₂ O, 20 μM DSS
Z-G4	TGGTGGTGGTGGTGGTGGTGGTGGTGTT	20 mM KPi, 70 mM KCl, pH = 7.0, 10%
		D ₂ O, 20 μM DSS
нт	TTGGGTTAGGGTTAGGGTTAGGGA	20 mM KPi, 70 mM KCl, pH = 7.0, 10%
		D ₂ O, 20 μM DSS

Supplementary Figures



Figure S1: Chemical structure of PhenDC₃



Figure S2: 1D ¹H NMR spectra showing **(A)** Non-binding of ligand PhenDC₃ to left-handed G4 (*Z-G4*) indicated by the unchanged spectrum and nonappearance of new peaks corresponding to complex formation upon titration of PhenDC₃ and **(B)** Binding of PhenDC₃ to parallel right- handed G4 (*T95-2T*), indicated by the gradual disappearance and appearance of the peaks corresponding to free form and complex form respectively. The black solid circles indicate the peaks corresponding to the G4 structure in its free form. The new set of peaks corresponding to the complex form of *T95-2T* with PhenDC₃ are marked with red asterisks. 2 µl of c.a. 20 mM solution of PhenDC₃ was added to the NMR tube containing 400 µl of 100 µM DNA samples.

Non-parallel right-handed G4 (HT)



Figure S3: 1D ¹H NMR spectra showing binding of PhenDC₃ to non-parallel right- handed G4 (*HT*), indicated by the partial disappearance and appearance of the peaks corresponding to free form and complex form respectively. The black solid circles indicate the peaks corresponding to the G4 structure in its free form. The new set of peaks corresponding to the complex form of *HT* with PhenDC₃ are marked with red asterisks. 2 µl of c.a. 20 mM solution of PhenDC₃ was added to the NMR tube containing 400 µl of 100 µM DNA samples.



Figure S4: 1D ¹H NMR spectra of the sequence **(A)** 2xBlock2 Δ and **(A)** T95-2T, with excess of the ligand PhenDC₃. CD spectra of the sequence **(C)** 2xBlock2 Δ and **(D)** T95-2T, with and without excess of the ligand PhenDC₃ demonstrating conformational transformation of left-handed G4 structure of 2xBlock2 Δ to parallel right-handed G4 on addition of ligand in excess whereas the parallel right-handed G4, T95-2T retains its structural conformation. 8 µl of ~20 mM solution of PhenDC₃ was added to the NMR tube containing 400 µl of 100 µM DNA samples.





Figure S5: (A) 23 amino acids peptide sequence of Rhau23. 1D ¹H NMR spectra showing (B) Non-binding of Rhau23 peptide to left-handed G4 ($2xBlock2\Delta$) indicated by the unchanged spectrum and nonappearance of new peaks corresponding to complex formation and (C) Binding of Rhau23 to parallel right- handed G4 (795-2T), indicated by the gradual disappearance and appearance of the peaks corresponding to free form and complex form respectively. The black solid circles indicate the peaks corresponding to the G4 structure in its free form. The new set of peaks corresponding to the complex form of T95-2T with Rhau23 are marked with red asterisks. 4.8 µl of c.a. 20 mM solution of Rhau23 peptide was added to the NMR tube containing 400 µl of 100 µM DNA samples.



Figure S6: Surface representation of the crystal structure of a reported left-handed G4 structure exhibiting <u>three</u> T-cappings on one of the outer tetrads and <u>four</u> T-cappings on the other tetrad.





Figure S7: Crystal structure of a reported left-handed G4 (pdb id: 7D5D) where spermine used in the process of crystallization of the DNA samples were found to be stacked on top of the exposed tetrads. Spermine being a linear molecule was able to reside in the cavity between the capping thymines, providing a potential approach to target left-handed G4s. The distance between the closest points of the adjacent capping thymines were measured to be c.a. 7.0 Å.