Supplementary Material (ESI) for Chemical Communications
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A mirror-image tetramolecular DNA quadruplex

Phong Lan Thao Tran,[a,b] Rui Moriyama,[c] Atsushi Maruyama,[c] Bernard Rayner,[a,b] and Jean-Louis Mergny[a,b]

Supporting Information

Experimental section

Oligonucleotide synthesis and purification

Unmodified oligonucleotides *D*-TG_4T, *D*-T_4G_4T and *D*-TG_4T with fluorescein at the 3' end were purchased from Eurogentec (Seraing, Belgium). Their concentrations were estimated using molar extinction coefficients provided by the manufacturer (57800 M^{-1} cm^{-1}, 82100 M^{-1} cm^{-1} and 78800 M^{-1} cm^{-1} at 260 nm, respectively)[1].

2'-Deoxy-*L*-guanosine and *L*-thymidine were prepared according to slightly modified procedures[2]. N-2-isobutyryl-2'-deoxy-*L*-guanosine and *L*-thymidine were dimethoxytritylated and further converted to the corresponding β-cyanoethylphosphoramidites according to standard procedures. *L*-oligonucleotides were synthesized on a Model 381A (Applied Biosystems Inc.) DNA synthesizer (1 μmol scale) using β-*L*-Thymidine 3'-lcaa CPG (ChemGenes) solid support for ***L*-TG$_4$T** and 3'-(6-FAM) CPG (Link Technologies) solid support for ***L*-TG$_4$T fluo**. Oligonucleotides were cleaved from the support and deprotected by treament with 30% aqueous ammonia for 5 hr at 55°C. Combined filtrate and washings were concentrated under reduced pressure, redissolved in water and the crude oligonucleotides were purified by reverse-phase HPLC on a Nucleodur 100-5 C18 ec column (Macherey Nagel) using a linear gradient of acetonitrile (5 to 22 % in 20 min) in 0.1 M triethylammonium acetate pH 7 at 1 ml/min flow rate. Fractions were collected and evaporated under reduced pressure. ms analyses were carried out on a MALDI-Tof-Tof mass spectrometer (Ultraflex, Brucker) operated in the reflectron mode. The matrix used was a 4:1 mixture of 2,4,6-trihydroxyacetophenone (10 mg/mL) in ethanol and 100 mM aqueous ammonium citrate. Samples (0.8μl of a 1:1 aqueous solution of oligonucleotide and matrix) were spotted on a stainless steel target and air-dried before analysis. For each sample, a MALDI mass spectrum was acquired by accumulating the ion signals from 100 UV laser shots with a constant laser power. *L*-TG_4T $[M-H]^-$: calc. 1862.3, found 1861.2; 3'-(6-FAM)-*L*-TG_4T $[M-H]^-$: calc. 2431.3, found 2430.6.

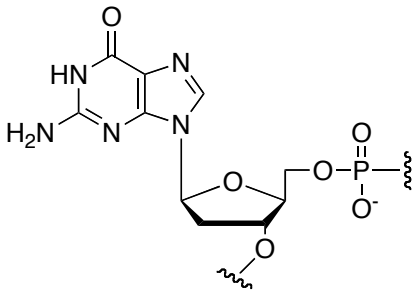

Figure S1. Structure of 2'-deoxy-*L*-guanosine -5'-phosphate as a constituent of *L*-DNA.

Cationic comb-type Copolymer (CCC).

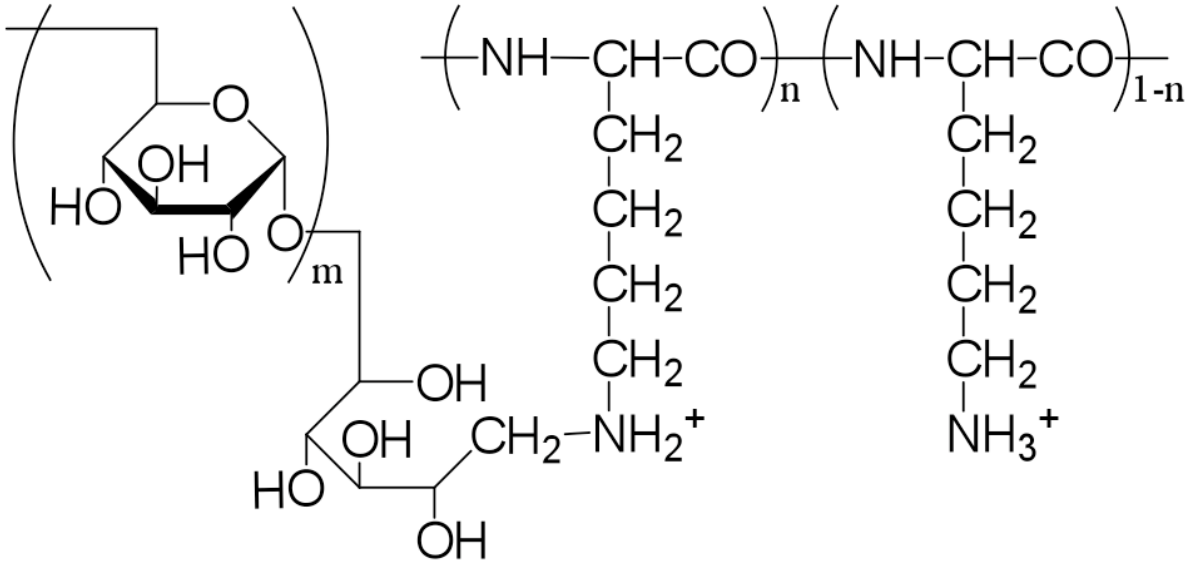

Figure S2. Structural formula of Cationic Comb-type Copolymer (CCC), poly(L-lysine)-*graft*-dextran[3]. MW of PLL backbone: 5.3×10^4, MW of dextran grafts: 8.4×10^3, grafting degree of Dex: 16 mol % (n = 0.16).

Absorbance differential spectra (TDS and IDS) and circular dichroism (CD)

Our reference conditions for this study were 10mM Lithium cacodylate pH 7.2 supplemented with 0.5M KCl. Oligonucleotides were prepared at 8 µ M strand concentration. Thermal difference spectra (TDS) were obtained by calculating the difference between the absorbance spectra recorded above and below the observed transition (data not shown). Isothermal difference spectra (IDS) were obtained by calculating the difference between the absorbance spectra of the folded and unfolded forms of a sample (after and before an isothermal kinetics experiment) (***Figure 1*** *left*). CD spectra were recorded on a JASCO-810 spectropolarimeter using a 1-cm path length quartz cuvettes as previously described[4].

UV melting experiments

Melting experiments were conducted as described earlier[5]. Denaturation was followed by recording the absorbance at 240, 260, 273 and 295 nm. Only apparent melting temperatures determined at 295 nm are shown in ***Figure 2*** and ***Table 1***. The experiments were performed in 10 mM Lithium cacodylate pH 7.2 supplemented with 0.1 or 0.5 M NaCl and with 10 µM or 18 µM strand concentration respectively. For the melting experiments in 0.1M NaCl, samples were pre-folded at 4°C for 48 hours and were heated from 10°C to 96°C, at a rate of 0.3°C/min. For melting experiments in 0.5M NaCl, samples were also pre-folded at 4°C for 48 hours and were heated from 10°C to 96°C, at a rate of 1°C/min.

Isothermal kinetics

These experiments were performed by starting from isolated strands and comparing association of *L*- and *D*- TG4T and *L+D* mixture by UV-isothermal experiments at 6°C in 10 mM Lithium cacodylate and 0.5 M KCl conditions as previously described[6,7]. Data was fitted using a model

previously published[6] and association rate constants (k_{on}) were calculated (***Table 1***). The corresponding order of the association reaction was assumed to be n = 4, as in previous studies. The sequences were tested at 8 µM strand concentration. Examples of isothermal kinetics are provided in the figure below:

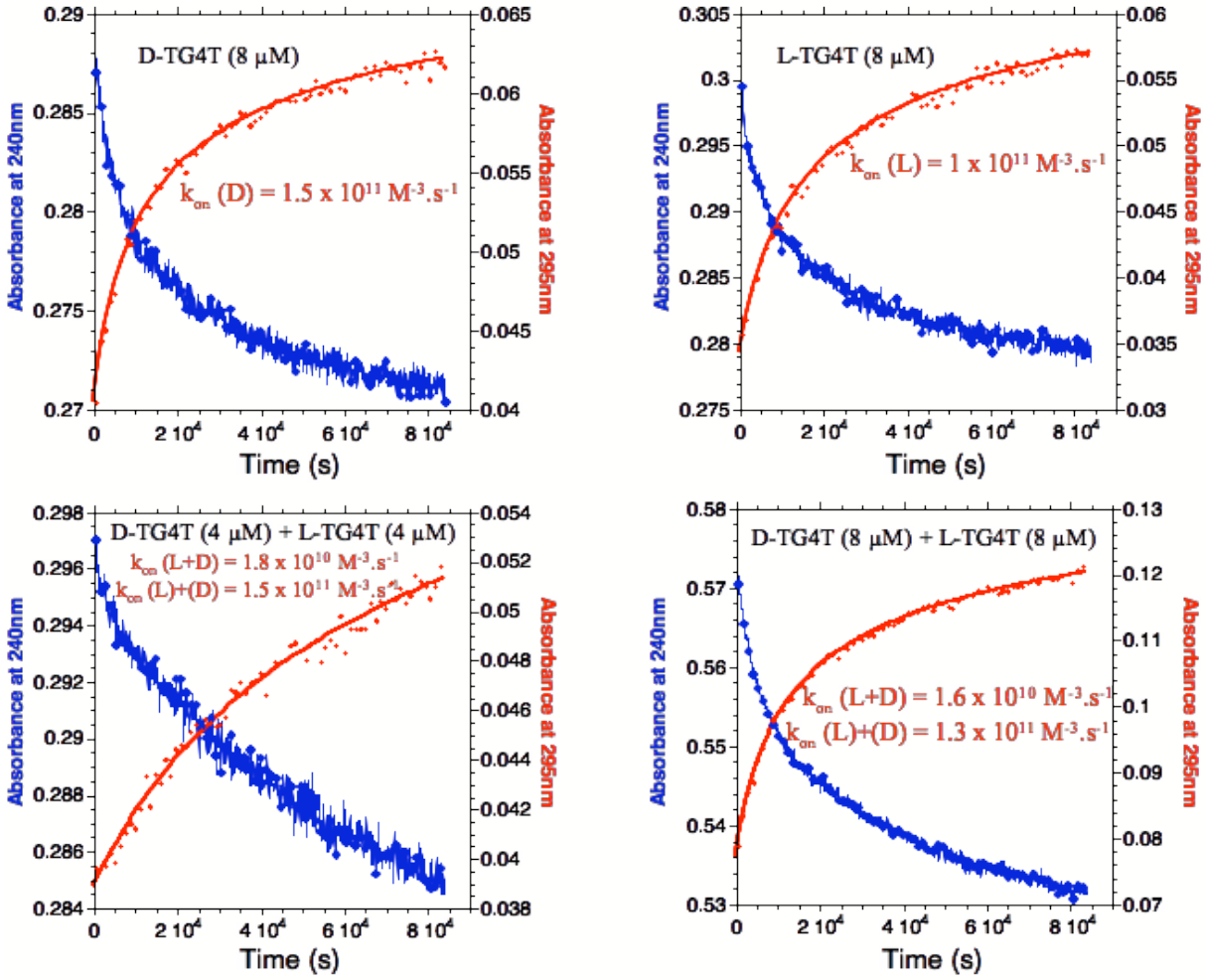

Figure S3. Example of isothermal experiments recorded at 240 nm (blue) and 295 nm (red). Top: left: *D*-TG_4T (8 µM) right: *L*-TG_4T (8 µM).
Bottom: Both *L*- and *D*- strands are present, each at 4 µM (left: total concentration 8 µM) or 8 µM (right: total concentration 16 µM). These samples indicate that the reaction proceeds at a much slower rate when having 4 µM of each strand, instead of 8 µM of one. In order to observe comparable rates, one must raise the concentration of each strand to 8 µM, as for the experiments with *L*- or *D*- alone.
The curves at 295 nm (red dots) were fitted using Kaleidagraph software with a mathematical function (red curve) leaving k_{on}, starting absorbance and final absorbance as floating parameters. The values provided in the main table are average ± SD of 3-5 experiments. Two fits are performed: one assumes that concentrations are additive while the other treat each enantiomer independently.

Non-denaturing gel electrophoresis

Non-denaturing gel electrophoresis allows separation of single-stranded oligonucleotides from tetramolecular G-quadruplex structures. Samples were loaded on a 20% polyacrylamide (acrylamide/bis-acrylamide 19:1) gel containing TBE 1X and KCl at 10 mM. Electrophoresis was performed at 4 W/gel to reach a temperature close to 35°C (electrophoresis performed at room temperature). To achieve complete quadruplex formation, samples (indicated by +) were incubated during 48 hours, at 4°C and at high strand concentration (200 µM), in 0.1M KCl and 10 mM LiCaco (pH 7.2). In parallel, samples (indicated by -) were diluted in water and heated 2 minutes at 90°C just before loading. Lithium cacodylate buffer was prepared by mixing cacodylic acid with LiOH. 12% glycerol was added just before loading. Bands were revealed by UV-shadow (60 µM strand concentration) using a UV light source (254 nm) and an ETNA-MS-ChemiBis digital camera (FSVT). This method does not require any labeling of any kind and relies solely on the absorbance of the nucleic acid in the far UV region (around 260 nm). We compared migrations of *L*- and *D*- sequences with or without cations.

L- or *D*-TG_4T and *D*-T_4G_4T were mixed in the same buffer (10mM LiCaco and 0.1M KCl) and incubated during 5 days at 4°C. (in ***Figure 3*** *left,* lane 4: 90µM *D*-T_4G_4T + 60µM *D*-TG_4T and lane 5: 180µM *D*-T_4G_4T + 90µM *D*-TG_4T is necessary to observe quadruplex formation). Oligothymidylate markers (dT_6 , dT_9 , dT_{18} , dT_{21} or dT_{24}) were also loaded on the gel. One should note that the migration of the dT_n oligonucleotides does not necessarily correspond to single strands [8]: these oligonucleotides were chosen here to provide an internal migration standard, not to identify intramolecular or higher-order structures.

Besides the gel shown in **Figure 3** *left*, we present here another independent experiment:

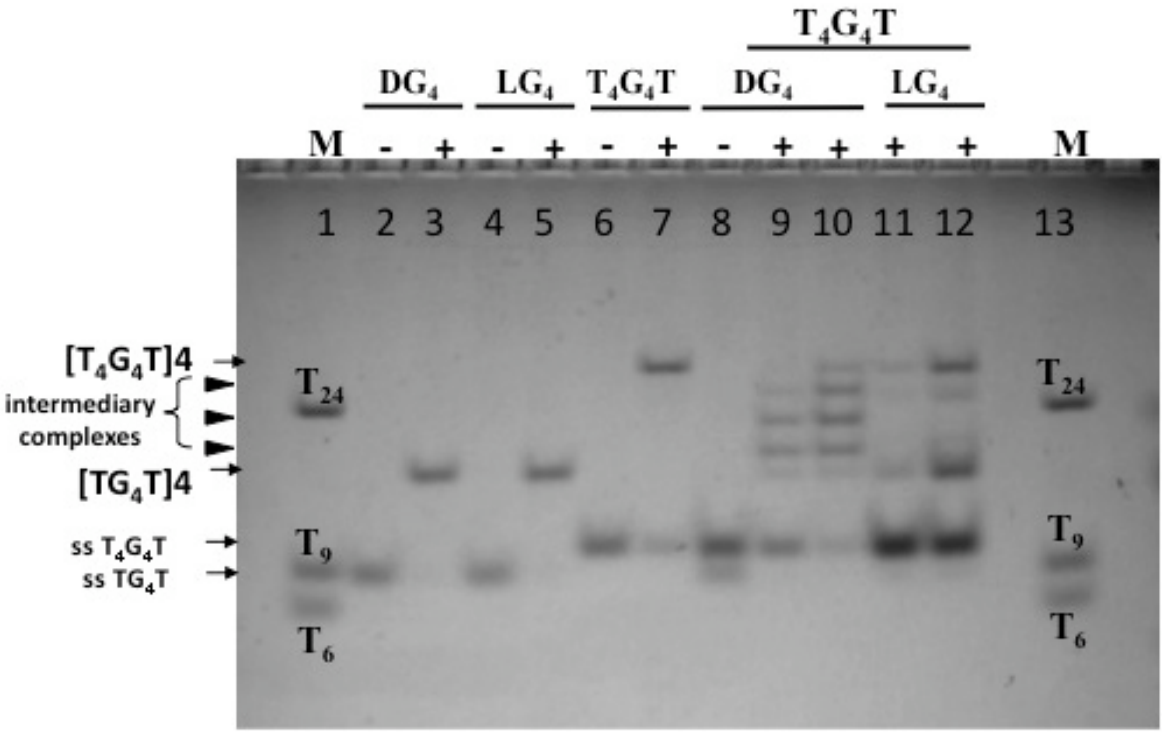

Figure S4. An independent gel experiment (the 5 leftmost lanes are shown in Figure 1). M corresponds to size markers. (+): conditions favorable to G4 formation; (-): conditions unfavorable to G4 formation (no cation and preheating). The gel was revealed by UV-shadow. Lanes 2-7: only one oligonucleotide is present, either *D*- or *L*-TG_4T or *D*-T_4G_4T. The difference in migration between the single strands is relatively modest (compare lane 6 with lanes 2 or 4) whereas the difference between the tetramolecular quadruplex is significant (compare lane 7 with lanes 3 or 5). Lanes 8-12: two oligonucleotides of different lengths are mixed. Lanes 9 and 10: the two D-strands are incubated together a two different concentrations. Besides a fraction of *D*-T_4G_4T that remain single-stranded, 5 bands are seen, corresponding to the "pure" *D*-$[T_4G_4T]_4$ and *D*-$[TG_4T]_4$ quadruplexes (slowest and fastest bands, respectively) and three intermediate complexes, in agreement with the formation of the tetramer. Lanes 11-12: when the *D*-T_4G_4T strand is mixed with *L*-TG_4T, the predominant species correspond to the "pure" *D*-$[T_4G_4T]_4$ and *L*-$[TG_4T]_4$ quadruplexes. A significant amount of *D*-T_4G_4T remains single-stranded. Lane 12: a minor band of intermediate mobility suggests that enantiomeric exclusion is not absolute.

Strand exchange reaction of intermolecular quadruplex by PAGE

L- or *D*-TG_4T with fluorescein at the 3' end (0.5 µM strand concentration) were incubated with an excess amount of preformed intermolecular DNA quadruplex, 20µM TG_4T, at room temperature, with and without 1 mM of cationic comb-type copolymer (CCC). The reaction was done in 10 mM LiCaco and 0.1 M NaCl. 12% glycerol and 5% PVS were added just before loading. Samples were loaded on a 20% polyacrylamide (acrylamide/bis-acrylamide 19:1) gel containing TBE 1X and NaCl at 10mM. Electrophoresis was performed at 4W/gel to reach a temperature close to 35°C (electrophoresis performed at room temperature). The gels were revealed by recording fluorescence at 520 nm (λ_{exc} = 480 nm).

The cationic comb copolymer (CCC) increased both the association and the dissociation rate of intermolecular quadruplex [9]. The copolymer acted as a nucleic acids chaperone that reduces the energy barrier need for both the dissociation and association folding [10] and enables the strand exchange reaction between the fluorescent strand and the preformed unlabeled quadruplex

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