Electronic Supplementary Material (ESI) for Chemical Communications


# Conformations of Individual Quadruplex Units studied in the Context of Extended Human Telomeric DNA

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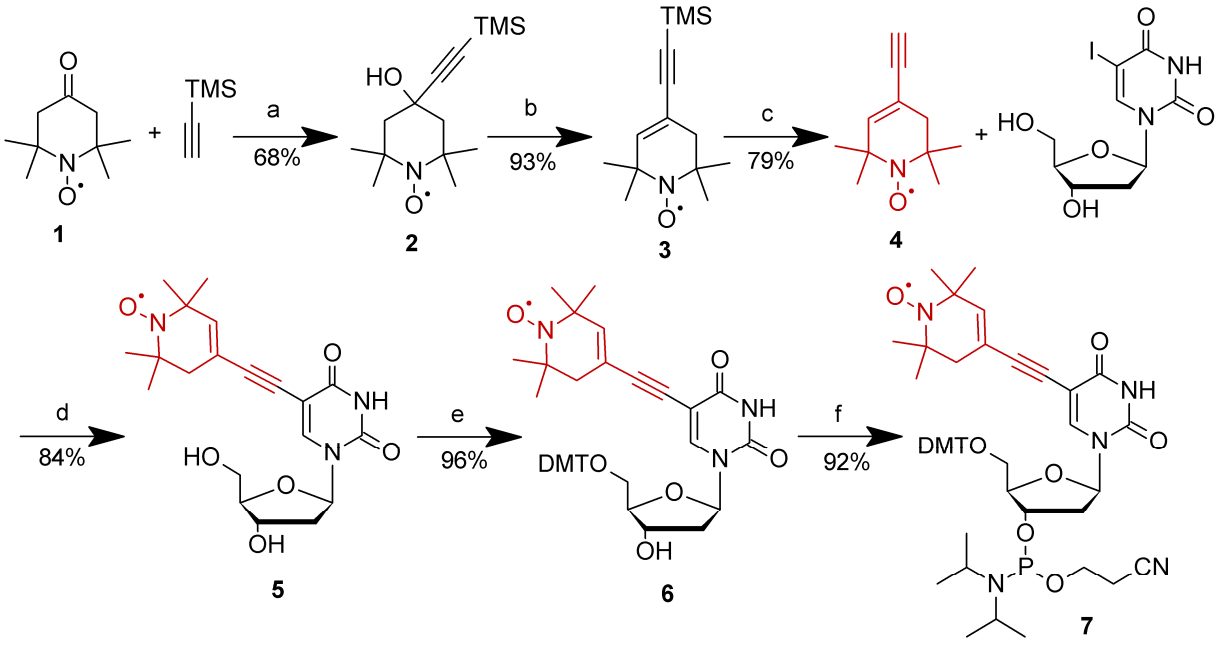
**Experimental Details:**

**Synthesis of Spin-labeled Phosphoramidite**

The spin labeled phosphoramidite **7** was synthesized from the commercially available nitroxide **1**. Compound **1** on condensation with lithium salt of trimethylsilylacetylene yielded the acetylenic alcohol **2**. The alcohol **2** on mesitylation followed by elimination furnished the TMS-enyne **3**. The TMS-enyne **3** on deprotection with tetrabutylammonium floride in wet THF yielded **4**. Compound **4** was then coupled with 5-iodouridine via Sonogashira coupling to furnish the nucleoside **5** which on protection with DMT-Cl at the 5' position and subsequent reaction with cyanoethyl diisopropylphosphoramidochloridite yielded the spin labeled phosphoramidite **7**.




*Reagent and Conditions*: a) nBuLi, anhyd. THF, 0°C to rt, 12h. b) MsCl, $Et_3N$, anhyd. $CH_2Cl_2$, 0°C to rt, 2-3h. c) TBAF, THF, rt, 1h. d) $Pd(PPh_3)_4$, CuI, $Et_3N$, DMF, rt, 12h. e) DMT-Cl, DMAP, Py, rt, 12h. f) $(iPr_2N)PCl(OCH_2CH_2CN)$, DIPEA, $CH_2Cl_2$, 0°C, 30 min.

**Oligonucleotide Synthesis**

The syntheses of DNA oligomers were performed on an ABI 394 DNA/RNA synthesizer with commercially available reagents from J. T. Baker and ABI using manufacturer supplied cycles and conditions. The spin-labeled DNAs were synthesized on 1.0 μmol scale (1000A° CPG columns) using phosphoramidites with standard protecting groups. The synthesis was performed with standard conditions, except for a longer coupling time for spin-labeled phosphoramidite (10 minutes total in several pushes). The DNA oligomers were concomitantly cleaved from the solid support and deprotected by 16 h treatment with concentrated aqueous ammonia at 55 $^{o}$C. The crude oligomers were concentrated on speed-vacuum and then purified twice by C18-RP-HPLC (0.1M TEAA/acetonitrile, pH 7.0), firstly with "DMT-on" and then with "DMT-off". The purified DNAs were lyophilized and then analyzed by ESI mass spectroscopy, double spin labeled DNA: 22555 (calc. 22564), single spin labeled DNA: 22392.8 (calc. 22402)

**DNA sequences used in this study**

(arrows indicate spin labels were attached on these particular nucleosides)

DSL-29-35 (for middle quadruplex unit)


DSL-5-11 (for terminal quadruplex unit)

**CD Spectra**

Sample preparation: 2.5 µM concentrations of DNA oligomers were prepared in water with 10 mM Tris-HCl (pH 7.5) and 100 mM KCl or NaCl. Annealing was performed by heating at 95 °C followed by slow cooling to 20 °C over 2 hours with the cooling rate 0.01°C per second. In order to reach maximum folding, annealed samples were incubated overnight at 4°C. The CD spectra were recorded on a Jasco 715 spectrometer in cuvettes with a 1 cm path length, resolution of 0.5 nm, band width of 1.0 nm and speed of 500 nm/min at 25 °C. Each spectrum was accumulated 5 times and averaged.

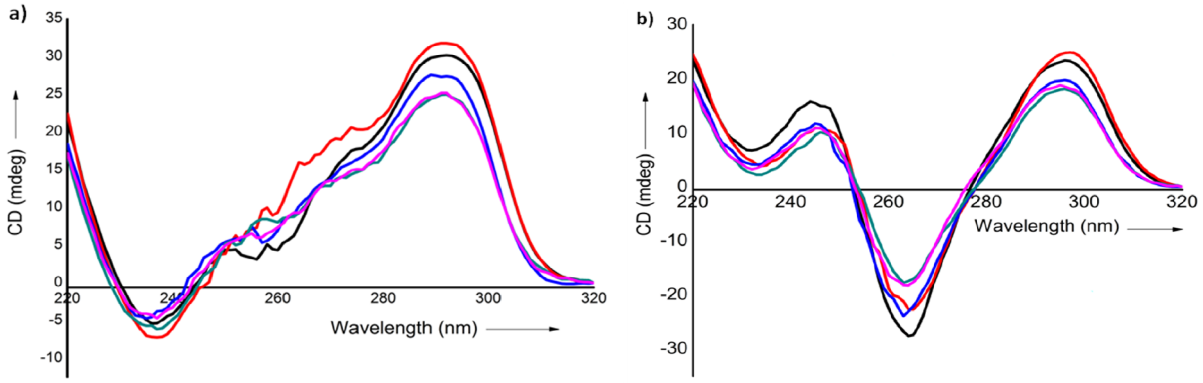



**Figure S1**: CD spectra of spin labeled and Unlabeled human telomeric DNA. 2.5 µM oligo in 10 mM Tris-HCl at 20 °C. a) 100 mM KCl, b) 100mM NaCl. Unlabelled HT-DNA (black), double spin labeled for middle quadruplex unit (red), single spin labeled for middle quadruplex unit blue), double spin labeled DNA for 5'-terminal unit (green), single spin labeled DNA for 5'-terminal unit (pink).

**EPR Measurements**

Sample preparation

40 µL of DNA helix in 10 mM Tris-HCl buffer (pH 7.5) was heated to 90 $^{o}$C and slowly cooled down to room temperature with the cooling rate 0.01$^{o}$C per second. 20% v/v glycerol was added to a final oligonucleotide concentration of 75 µM. The samples were transferred into 3 mm (outer diameter) quartz tube, shock frozen in liquid nitrogen and put into the spectrometer.

EPR experiments

Utilizing double labeled sequences, short contributions below 1.5 nm in the distance distributions were excluded by cw-EPR measurements at T = 120 K by comparison with single spin-labeled sequences. All DEER experiments have been performed at T = 45 K in X-band using a Bruker Elexsys E580 equipped with a split-ring resonator. The magnetic field and the pump frequency were adjusted such that the pump π-pulse (length: 12 ns) was applied to the maximum intensity band of the nitroxide spectrum and in the center of the resonator mode. The observer frequency was increased by 67.2 MHz with respect to the pump frequency. Pulse lengths of the observer channel were 16 and 32 ns for π/2- and π-pulses, respectively. A phase cycle (+x)-(−x) was applied to the first observer pulse. The complete pulse sequence is given by: $\pi/2_{obs}$-$\tau_1$-$\pi_{obs}$ -t-$\pi_{pump}$-($\tau_1$+$\tau_2$-t)-$\pi_{obs}$-$\tau_2$-echo. The DEER time-traces for ten different $\tau_1$-values spaced by 8 ns starting at $\tau_1$=200 ns were added in order to suppress proton modulations. Typical accumulation times per sample were 10 hours.

Analysis of DEER traces

In order to analyze the data and extract the distance distributions, the software package DeerAnalysis2010 has been used [G. Jeschke, V. Chechik, P. Ionita, A. Godt, H. Zimmermann, J. Banham, C. R. Timmel, D. Hilger, H. Jung, *Appl. Magn. Reson.* 2006, *30*, 473-498. G. Jeschke, *Chemphyschem* 2002, *3*, 927-932. G. Jeschke, A. Koch, U. Jonas, A. Godt, *J. Magn. Reson.* 2002, *155*, 72-82. G. Jeschke, *Macromol. Rapid Comm.* 2002, *23*, 227-246.]. Experimental background functions were derived from individually measured DEER traces of corresponding singly labeled sequences. The background functions are similar to a background model of a homogeneous three-dimensional distribution excluding intermolecular quadruplex

formation. The errors of the distance-distribution parameters were determined by changing parameters and inspection of the agreement with the experimental DEER time traces. The range of parameters that gives acceptable fits is given as the error margin of the parameters

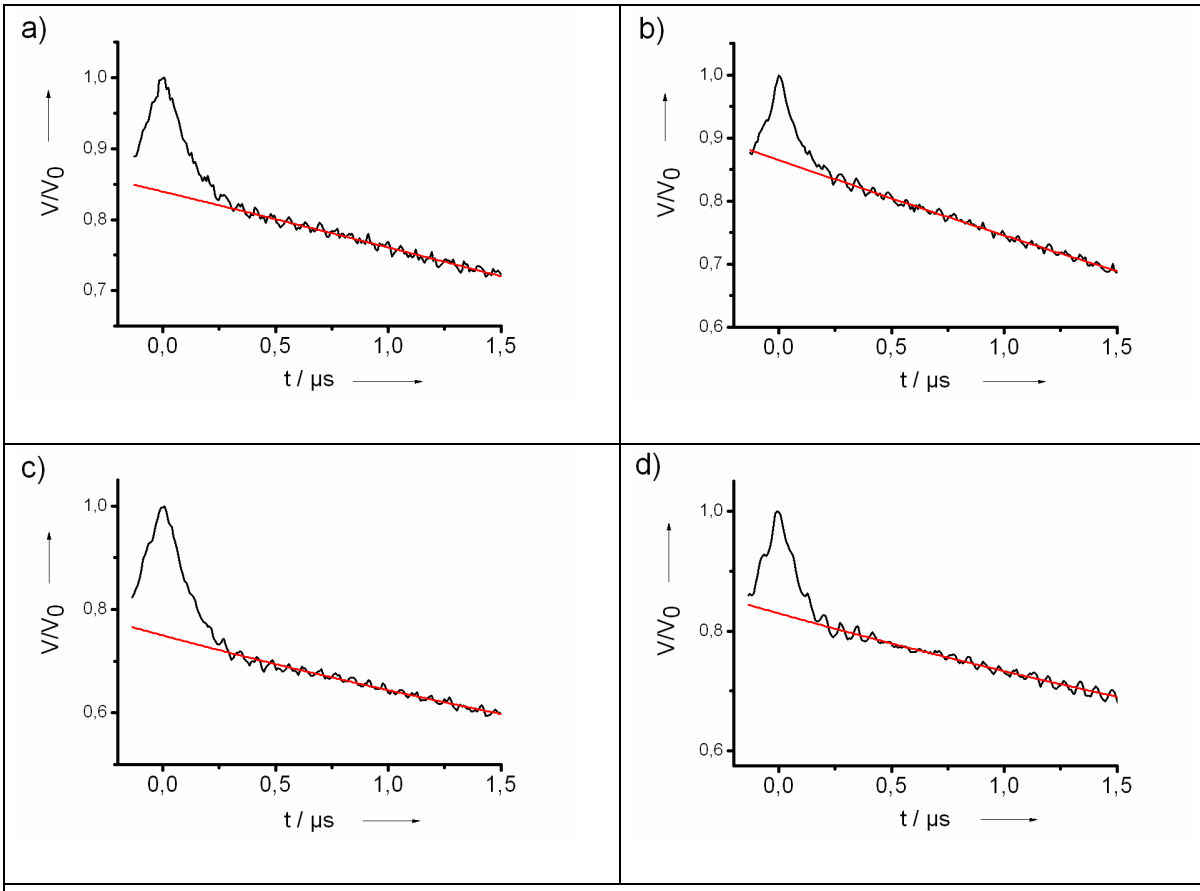



Figure S2. Dipolar evolution prior to background correction (black) and experimentally derived background function (red) shown for the long HT-DNA labeled in the middle quadruplex-forming unit in $Na^+$ (a) and $K^+$ (b) as well as for the long HT-DNA labeled at terminal quadruplex-forming unit in $Na^+$ (c) and $K^+$ (d) solutions.

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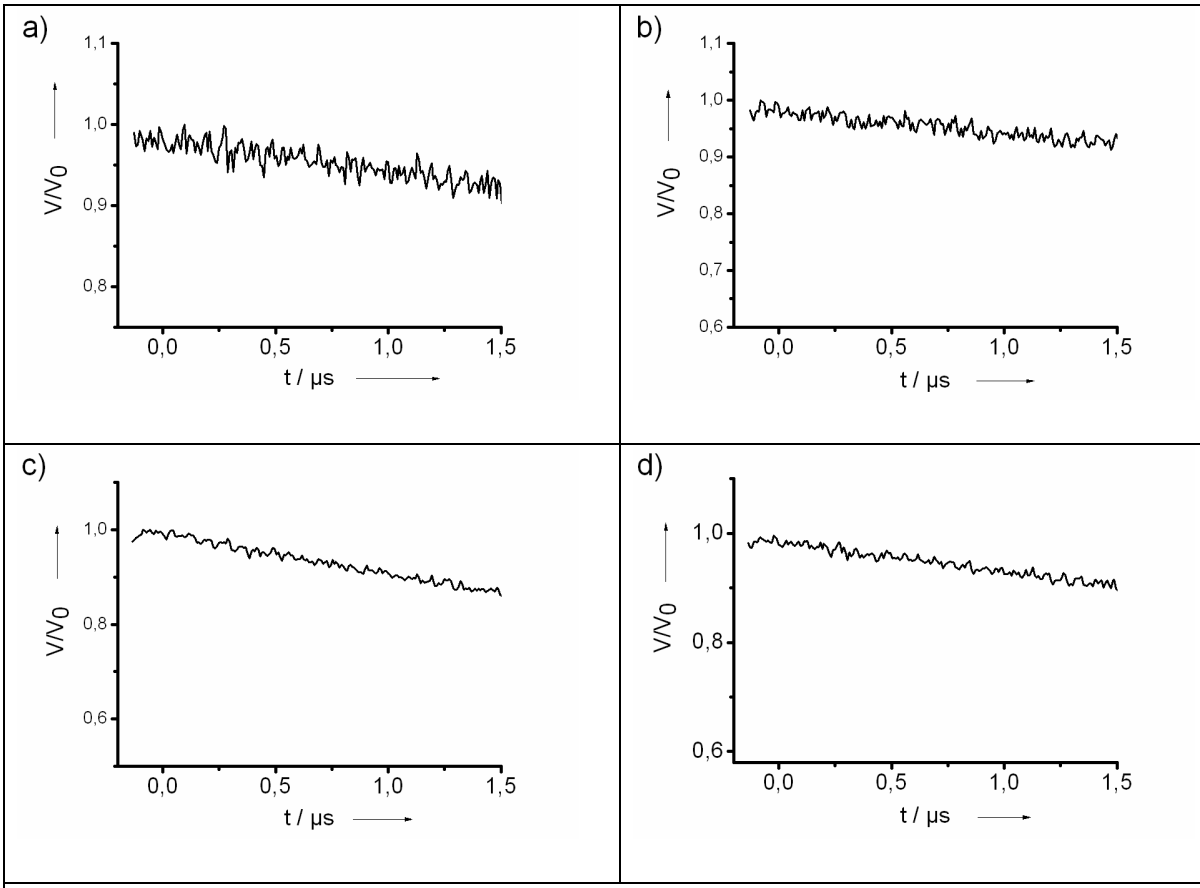


Figure S3. Dipolar evolution measured for the singly spin labeled long HT-DNA. Labeled in the middle quadruplex-forming unit in $Na^+$ (a) and $K^+$ (b) as well as for the long HT-DNA labeled at terminal quadruplex-forming unit in $Na^+$ (c) and $K^+$ (d) solutions.