Supporting Information

Label-free probing of G-Quadruplex formation by Surface-Enhanced Raman Scattering

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Synthesis of DNA

The synthesis of unmodified oligodeoxynucleotides (ODNs) was performed using the standard phosphoramidite chemistry on controlled pore glass supports using an Expedite 8909 DNA synthesizer (15 μmol) (Applied Biosystems). The synthesis of tetra-end linked oligodeoxynucleotides (TEL-ODNs), L-$(TG_4T)_4$ and S-$(TG_4T)_4$, was performed according to the previously described procedure. [1,2] After automated synthesis, the oligonucleotides were detached from the support, deprotected, and purified by HPLC on an anion exchange column (Macherey Nagel Nucleogel Sax 1000-8), followed by desalting on a molecular exclusion column (BIORAD Biogel P-2 fine). The oligonucleotides were then characterized by MALDI-TOF mass spectrometry using a Bruker Autoflex instrument. Stock solutions of each oligonucleotide were prepared in double distilled water and their concentration calculated by UV absorbance using the coefficient of molar extintion $\varepsilon_{260}(M^{-1}cm^{-1})$ reported in table 1.

Table 1: DNA sequences used in this study.

Sequence	$\varepsilon_{260}(M^{-1}cm^{-1})$	Mass spectrometry results	
		Calculated (MW)	found
$d(TG_3T)$	47700	1534.1	1533
$d(TG_4T)$	57800	1863.2	1862
$d(TG_5T)$	67900	2192.4	2191
$L\text{-}(TG_4T)_4$	231200	8815.7	8814
$S\text{-}(TG_4T)_4$	231200	8220.9	8220

Formation of G-quadruplex structures

The ODN samples were dissolved in 100 mM potassium buffer (10 mM KH_2PO_4, 90 mM KCl, pH = 7.0) and the corresponding quadruplexes were formed by heating at 90°C for 5 min and slowly cooled at room temperature for 12 h. The solutions were then equilibrated at 4°C for 24 hours before data acquisition.

Circular Dichroism (CD)

CD spectra of ODNs (20 μM) were measured by using a Jasco J-715 spectropolarimeter equipped with a Jasco JPT-23-S temperature controller. Spectra were recorded in the 220-360 nm range, using 1 mm path-length cuvettes. Spectra were averaged over 5 scans, which were recorded at a scan rate of 100 nm/min with a response time of 1 s and a bandwidth of 1 nm. Buffer baseline was subtracted from each spectrum and the spectra were normalized to have zero ellipticity at 360 nm. CD-melting experiments were performed in a temperature range of 20-90° C by monitoring the absorbance at 264 nm, at a 0.5 ° C/min heating rate. The melting temperatures were calculated as previously reported by Mergny et al.[3]

Experimental set-up

SERS analyses were performed by using a home-made system based on an inverted microscope endowed with a Raman probe at 532 nm (Spectra Physics, Millennia Xs).[4] This laser beam was

tightly focused into the sample by an Olympus oil-immersion infinity corrected objective lens (Olympus 100X, 1.4 N.A.). Typically, the laser power impinging on the sample was around 50 µW. Scattered light from the sample followed back the same path as the incident Raman probe, reaching a holographic notch filter, where radiation at 532 nm was removed. The so-filtered radiation was focused onto the entrance slit (set at an aperture of 50 mm) of the spectrometer (TRIAX 180, Jobin-Yvon), equipped with a 1800 lines/mm holographic grating. The Raman radiation was finally detected by using a back-illuminated CCD (Pixis 100, Princeton Instruments), thermoelectrically cooled at -70°C. The CCD was interfaced to a personal computer, where spectra were stored and analyzed. CCD spectral calibration was performed by acquiring the Raman signal of polystyrene latex beads (SERVA Electrophoresis), whose Raman peaks positions are accurately known.[5] The measured spectral resolution is 2 cm^{-1}, as estimated from the polystyrene spectrum by measuring the FWHM of the 1001.4 cm^{-1} peak. Samples to be analyzed were placed in a chamber constituted by two 150 µm glass coverslips (Knittel Glasser, thickness no.1), sealed with parafilm stripes. To perform SERS measurements, an aliquot of Ag-colloidal solution was added to the sample.

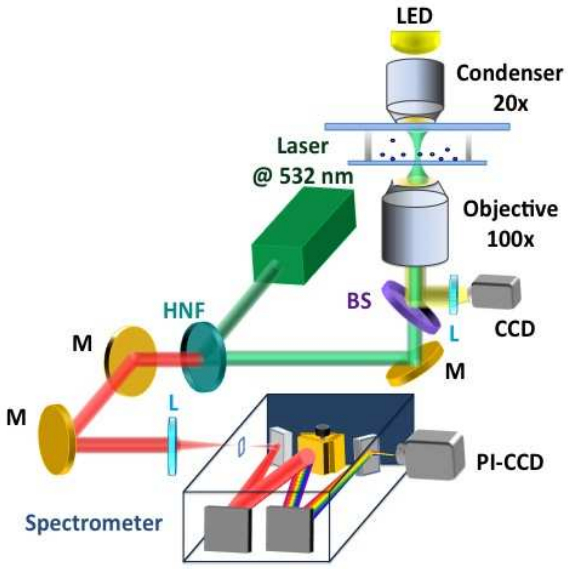

Figure 1: Schematic of the experimental set-up used for SERS analysis. Abbreviations: M, mirror; L, lens; HNF, holographic notch filter; BS, beam splitter; CCD, Charge Coupled Device.

Analysis of SERS spectra fluctuations

We observe for d(CTGTGTT) a relative enhancement of the Raman band around 1086 cm^{-1} associated with the phosphate backbone, which indicate the proximity of backbone to the SERS substrates. Nevertheless, once more we have found that SERS spectra assume a much higher degree of reproducibility in K^+ buffer (see Figure 2). This finding is clearly correlated to the higher order of the DNA strands.

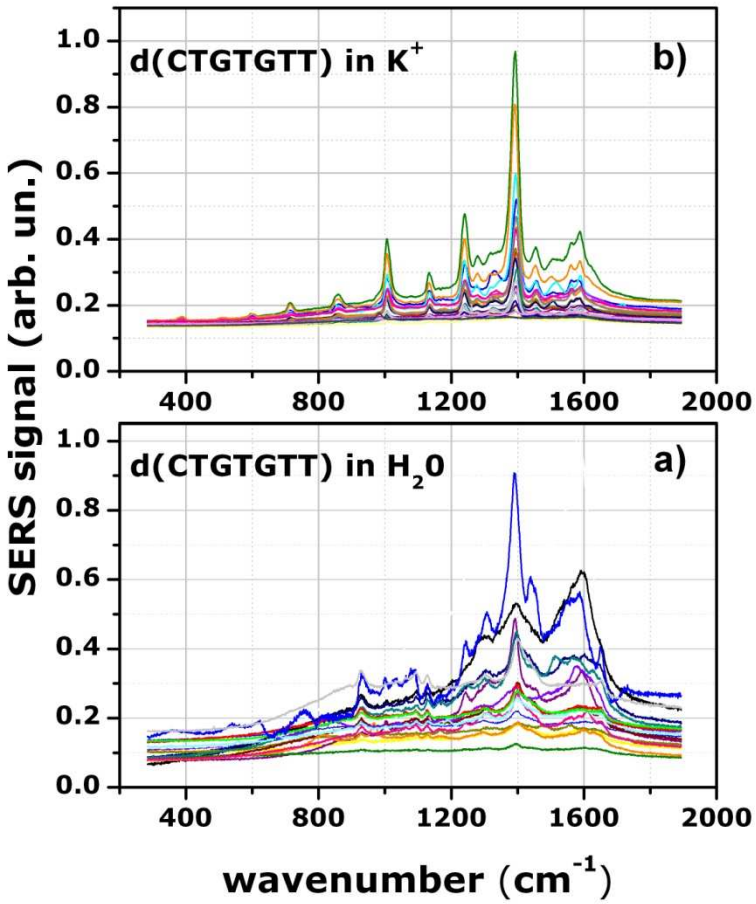

Figure 2: 20 randomly selected SERS acquisitions (row data) for d(CTGTGTT) DNA strands, at a concentration of 10^{-5} M, in H_2O (part a) and in the K^+ buffer (part b).

References

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