Electronic Supplementary Information
Crowding and Conformation Interplay on G-Quadruplex Human DNA by Ultraviolet Resonant Raman Scattering

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Quantum chemical computations

Figure S11 reports the simulated Raman activity (RA) and intensity for the ground state molecular structure of deoxyguanosine in the *syn*-conformation (magenta) and *anti*-conformations (cyan).

Table S4 reports harmonic frequencies (RA>10) for the ground state of deoxyguanosine in *syn*- and *anti*-conformations.

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Circular dichroism

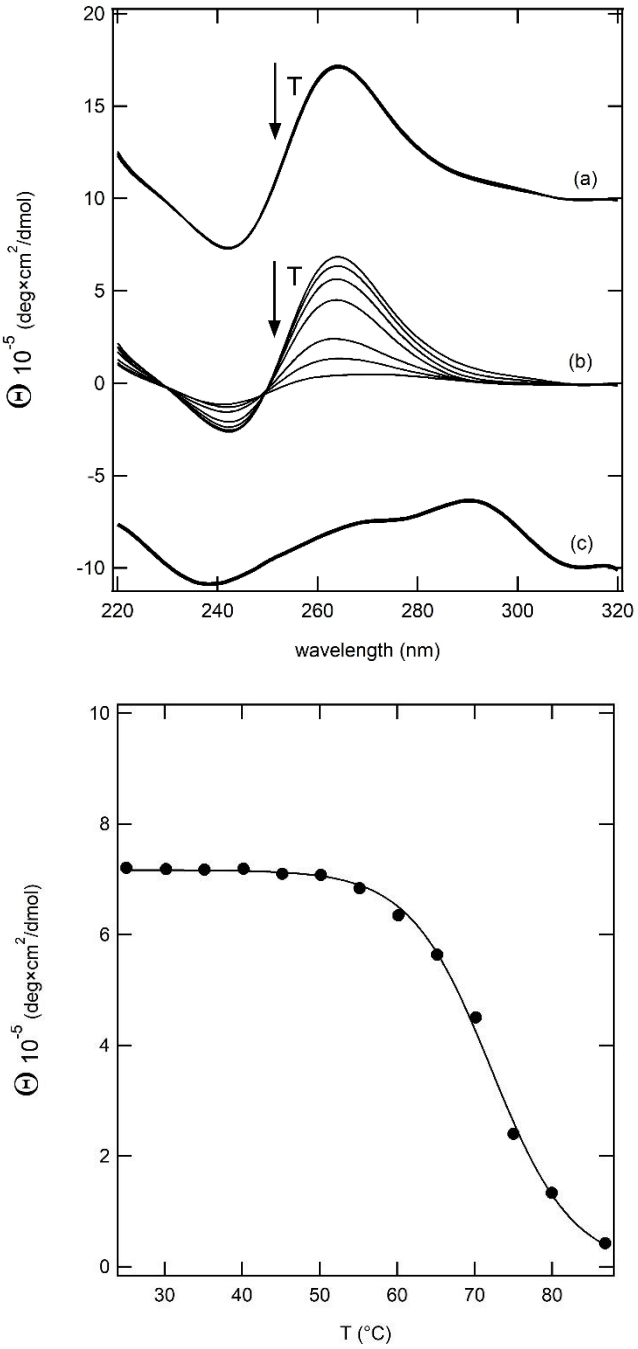

Figure S1: Upper panel**:** CD spectra for SC during a 25 – 85 °C temperature scan (5 °C step, 1 °C/min rate, 0.5 mm cell path length). Arrows indicate temperature increase. All the spectra have been corrected for buffer baseline. CD spectra were smoothed by applying a Savitzky-Golay filter. Groups of curves have been shifted in the vertical axis by 10 units.
(a) 25 - 50 °C, (b) 55 – 85 °C, (c) spectrum at 25 °C at the end of the temperature scan.
Lower panel: CD melting profile at 264 nm, T_m = 72 °C.

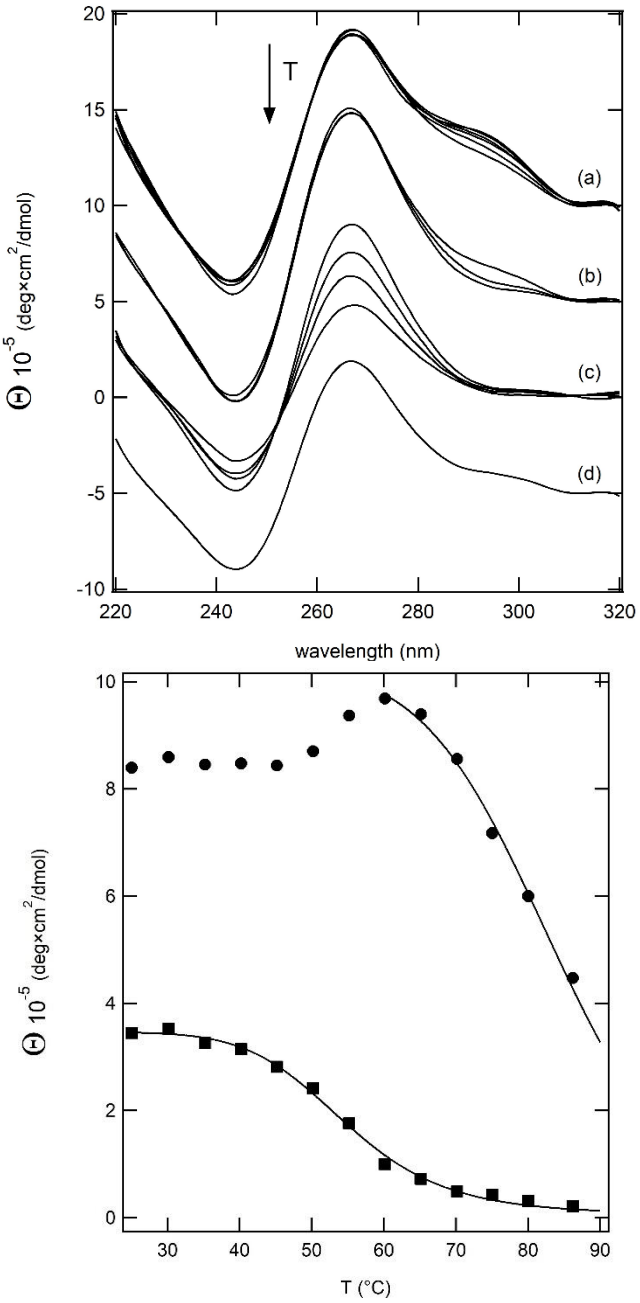

Figure S2: Upper panel**:** CD spectra for sample PC during a 25 °C – 85 °C temperature scan (5 °C step, 1 °C/min rate, 0.1 mm cell path length). Arrows indicate temperature increase. All the spectra have been corrected for buffer baseline. CD spectra were smoothed by applying a Savitzky-Golay filter. Groups of curves have been shifted in the vertical axis by 5 units. (a) 25 – 50 °C, (b) 55 – 65 °C, (c) 70 - 90 °C, (d) spectrum at 25 °C at the end of the temperature scan.
Lower panel: CD melting profiles at 264 nm, T_m > 84 °C (circles); CD melting profiles at 295 nm, T_m= 57 °C (squares).

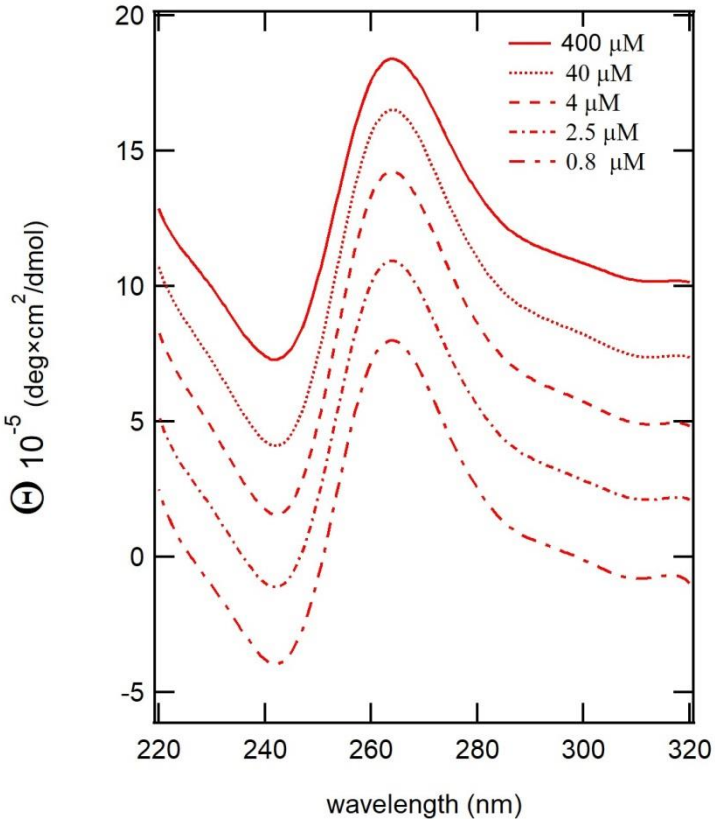

Figure S3: CD spectra at 25 °C of SC as a function of concentration showing unaltered conformation topology with buffer dilution. Curves have been shifted in the vertical direction by 2.5 units. CD spectra were smoothed by applying a Savitzky-Golay filter. Cell path length was 0.5 mm for 400 μM and 40 μM, 10 mm for 4 μM, 2.5 μM and 0.8 μM.

Polarized Ultraviolet Resonant Raman Scattering

VV and HV-polarized UVRR spectra for samples under NC and SC conditions were acquired every 5 °C during the temperature scan from 25 to 90 °C at the scanning rate of approximately 0.05 °C min^{-1}. VV and HV-polarized UVRR spectra for the sample under PC conditions were acquired with variable step, i.e. every 5 °C from 25 to 50 °C and every 10 °C from 50 to 90 °C. The acquisition time at a given temperature for each polarization was 90 min. The resulting underlying scanning rate were 0.025 °C min^{-1} and 0.05 °C min^{-1} in the two temperature ranges from 25 °C to 50 °C and from 50 °C to 90 °C, respectively . Temperature stability was less than ±0.1 °C.

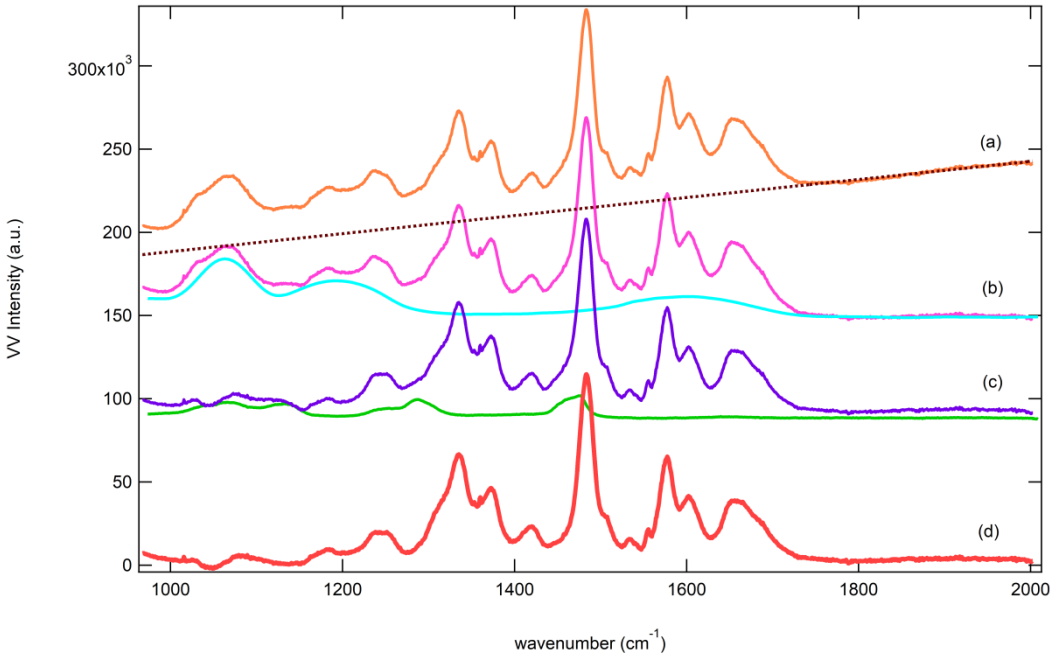

Figure S4: example of steps for data reduction, here applied to VV-polarized UVRR spectrum for PC buffer solution at 25 °C. Correction steps have been applied to the averaged and spikes-removed spectra, after subtracting the dark signal to each acquired spectrum. The wavenumber axis has been calibrated with known standards.

step (a) - Fluorescence background subtraction from sample and corresponding buffer solutions (data not shown).

The fluorescence background (dotted line) has been evaluated by fitting the region above 1800 cm^{-1} with a straight line (or a low order polynomial function). The same procedure has been applied for the corresponding spectrum of pure buffer.

step (b) – cell subtraction from the sample and corresponding buffer solutions (data not shown).

After subtracting the fluorescence background (magenta profile), the contribution of the empty cell (represented in cyan) has been rescaled by assuming that the shallow contribution around 1000 cm^{-1} is uniquely due to the cell and subtracted.

step (c) – buffer subtraction.

The relative contribution from the buffer has been evaluated in the range around 3200 cm^{-1} (OH stretching contribution, not shown) by imposing that the OH stretching contribution from the signals acquired from buffer and sample do coincide (valid for diluted samples). The obtained scaling factor has then been applied to the UVRR of the buffer signal (green profile).

The final spectrum is represented in red by curve (d).

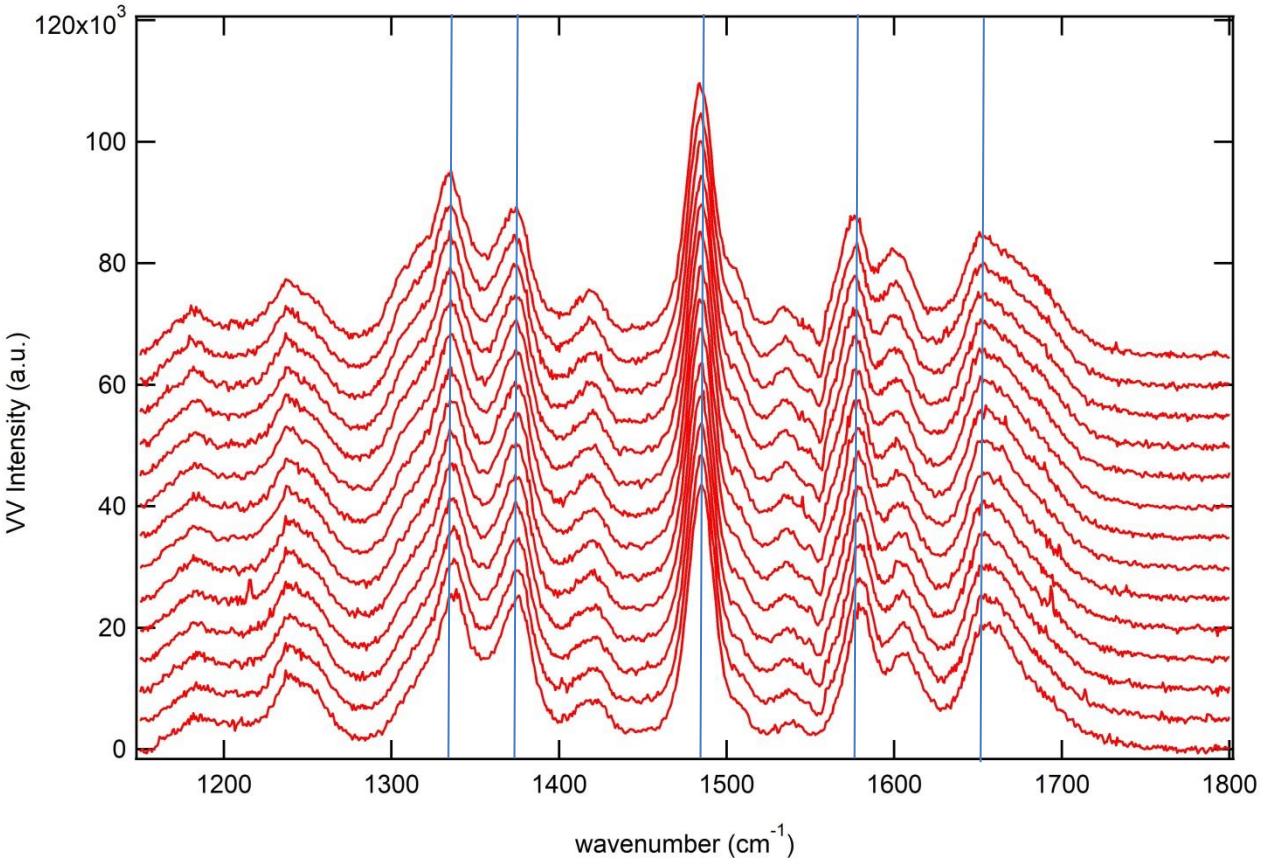

Figure S5: VV polarized Ultraviolet Resonant Raman spectra during a temperature scan in the 25 – 90 °C range (temperature step 5 °C) for SC. The temperature increases from bottom to top. Each VV- or HV- (data not shown) spectrum represents the average of 2 spectra of 15 minutes duration. Data have been shifted in the vertical direction by 500 units for each 5 °C step. Vertical blue lines show band shifts.

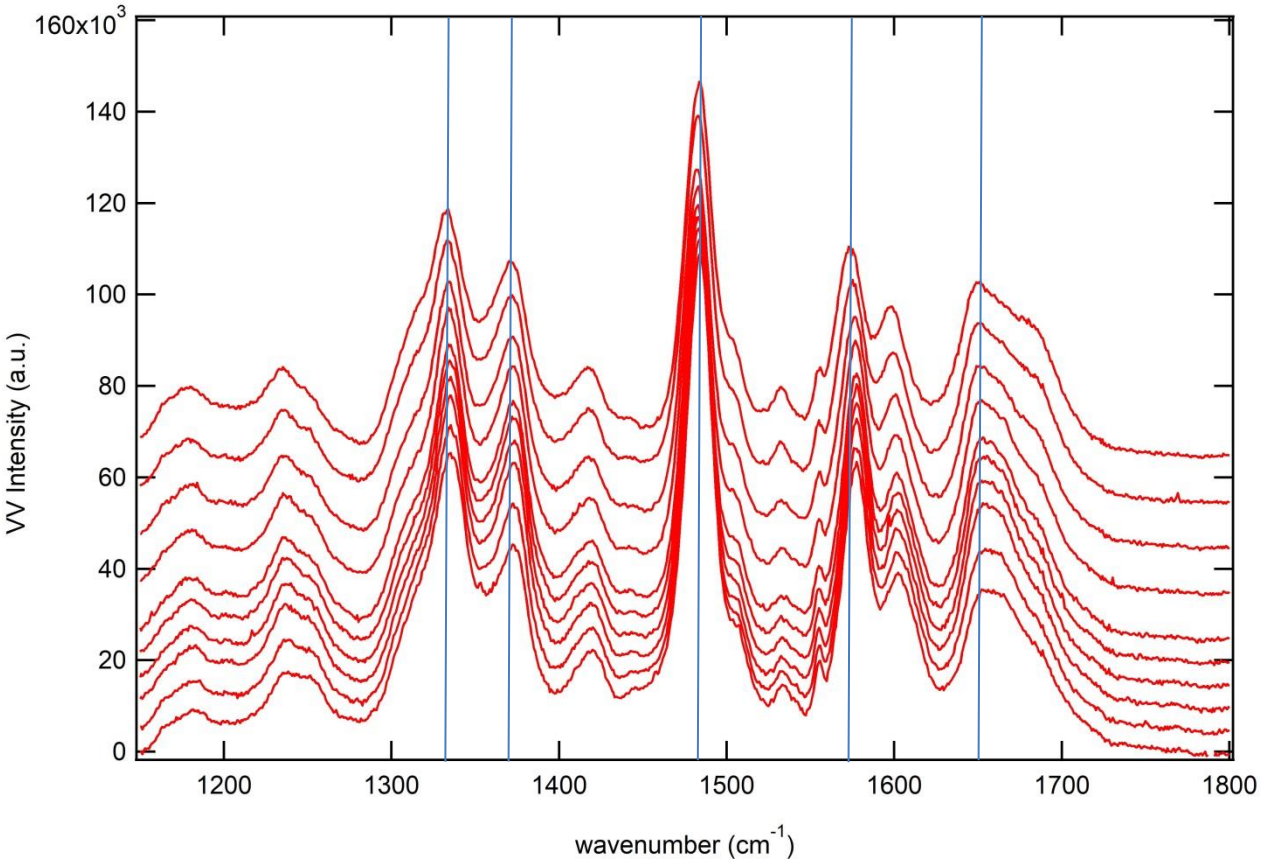

Figure S6: VV polarized Ultraviolet Resonant Raman spectra during a temperature scan of PC. Temperature increases from bottom to top: 25, 30, 35, 40, 45, 50, 60, 70, 80, 90°C. Data have been shifted by 500 (each 5 °C step) or 1000 units (each 10 °C step). Each VV- or HV- (data not shown) spectrum represents the average of 6 spectra of 15 minutes duration. Vertical blue lines show band shifts.

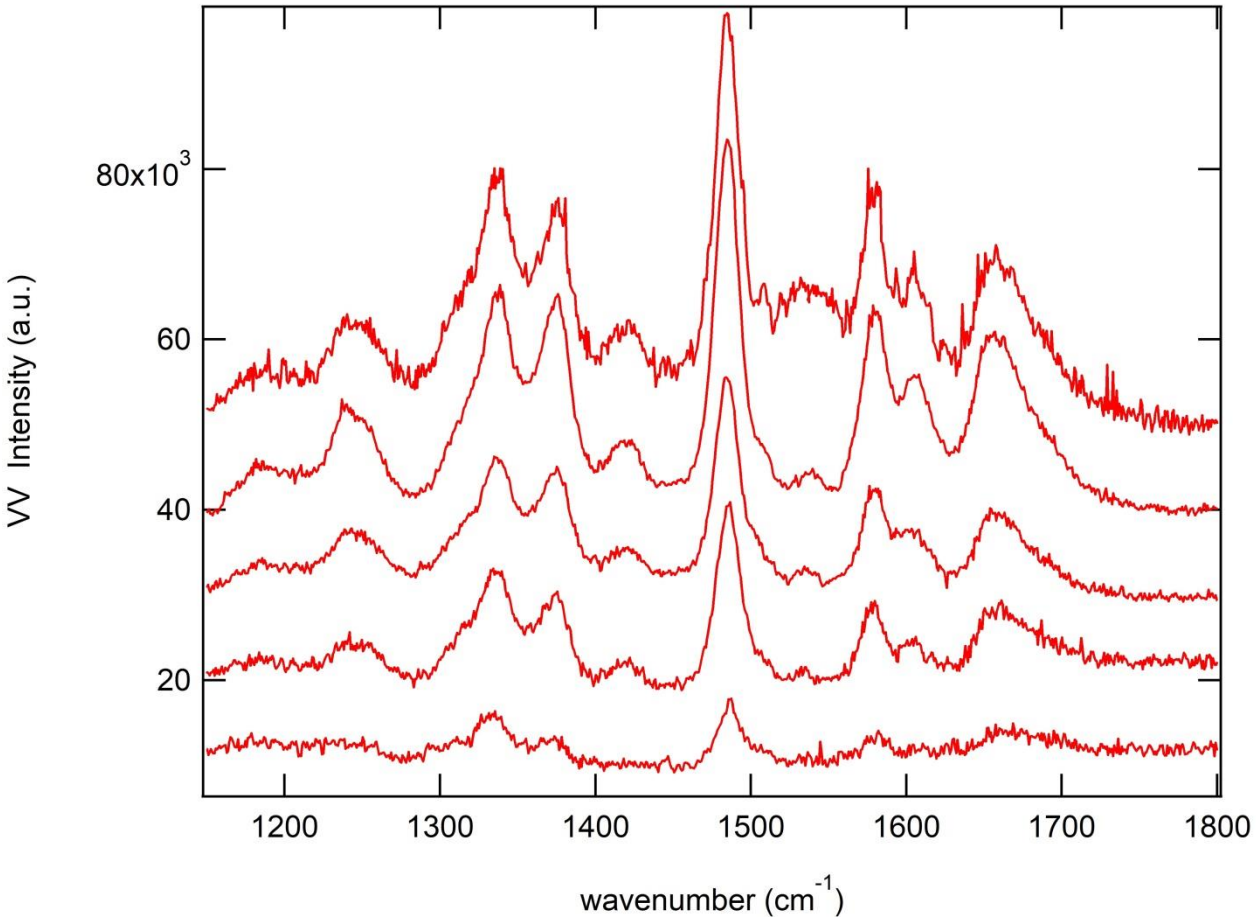

Figure S7: VV polarized Ultraviolet Resonant Raman spectra at 25 °C for SC solutions at different concentrations. From top to bottom: 400 μM and 40 μM, 4 μM, 2.5 μM and 0.8 μM. Data are the average of several spectra of 15 minutes duration and have been shifted for better clarity. Depending on the signal to noise ratio, each VV- or HV- (data not shown) spectrum has been acquired with variable acquisition time, i.e. 90 minutes for 400 μM, 30 min for 40 μM, 120 min for 4 μM, 810 min for 2.5 μM and 0.8 μM. The unaltered position of the Hoogsteen peak shows that self-crowding conformation is preserved upon dilution.

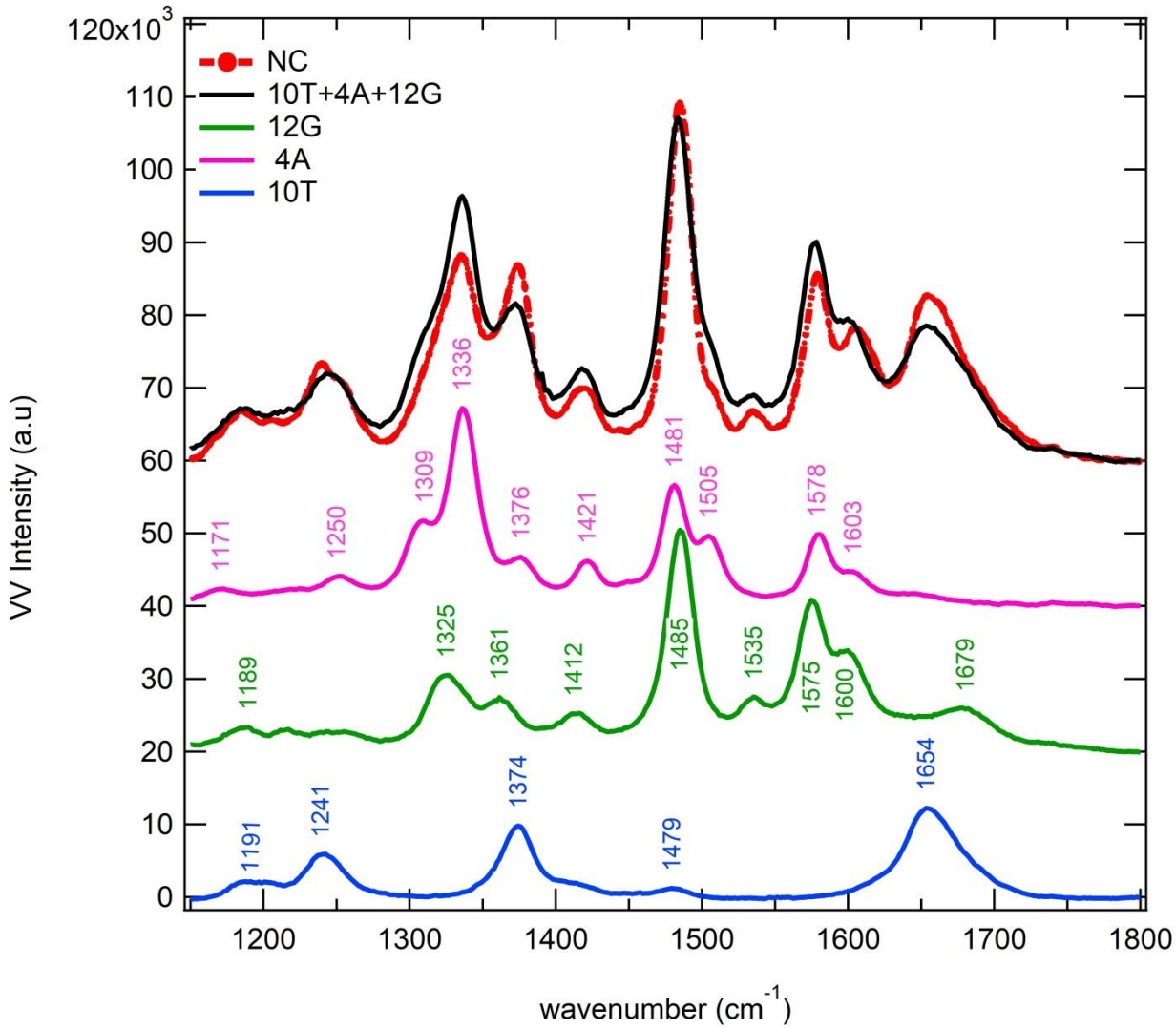

Figure S8: VV-polarized UVRR Spectra at 25 °C for 40 μM solutions of NC tel-26 G4 [TTAGGG TTAGGG TTAGGG TTAGGG TT] (red dots) and for the indicated deoxynucleosides triphosphate components (magenta A; green G; blue T), whose relative intensity has been weighted taking into account their contribution in tel-26 (10 T + 4 A + 12 G). The black spectrum is the sum of these latter components. All solutions were prepared by dissolving the solute in 10 mM potassium phosphate, 100 mM KCl, 0.1 mM EDTA at pH 7.0.

Data analysis and fitting

Data analysis and fitting in the framework of the Kubo Anderson model.

The scattered light from the sample polarized parallel (I_{VV}) and perpendicular (I_{HV}) with respect to the incident radiation was collected. Isotropic and anisotropic Raman intensity profiles were obtained according to the relation:

$I_{iso} = I_{VV} - 4/3\ I_{HV}$ and $I_{aniso} = I_{HV}$ [1]

The spectral profiles were fitted by a Kubo Anderson function (KAF).[1] Under this framework, the isotropic spectral shape for N vibrations can be represented by

$$I_{iso} = \sum_{i=1}^{N} A_i K_i\left(\omega, \omega_{0,i}, \sigma_i^2, \alpha_i\right) + B \quad [2]$$

where B is a constant background, A_i are scaling factors, and $K_i\left(\omega, \omega_{0,i}, \sigma_i^2, \alpha_i\right)$ is the KAF which can be expressed by the following equation

$$K_i\left(\omega, \omega_{0,i}, \sigma_i^2, \alpha_i\right) = \frac{e^{{\alpha_i}^2}}{\sigma_i} \sum_{n=0}^{\infty} \frac{(-{\alpha_i}^2)^n}{n!} \frac{\alpha_i + n/\alpha_i}{(\alpha_i + n/\alpha_i)^2 + \frac{(\omega-\omega_{0,i})^2}{{\sigma_i}^2}} \quad [3]$$

where σ_i^2 is the frequency second moment and its square root describes the frequency distribution of the active oscillator around the unperturbed frequency $\omega_{0,i}$, and $\alpha_i = \sigma_i \tau_{c,i}$ is a parameter which describes the modulation regime of the dephasing correlation time [2], and $\tau_{c,i}$ is the environmental modulation time of the perturbative events. This model predicts that if α <<1, the modulation of the probe molecule is fast compared to the molecular perturbations of the system in which it is embedded, and the original phase of the modulation is kept unaltered for a long time. In this particular case the KAF equation reduces to a Lorentzian function. Under these circumstances the vibrational dephasing relaxation time, τ_{vibr}, can be calculated as:

$$\tau_{vibr} \cong \frac{1}{\sigma^2 \tau_c} = \frac{1}{\pi c \Gamma_{iso}} \quad [4]$$

where c is the speed of light and Γ_{iso} is the full width at half maximum of the Lorentzian for isotropic intensity mode.

The non coincidence effect, NCE, is defined as the difference of the position of the maxima of the isotropic and anisotropic Raman components:

$$\Delta\nu_{NCE} = \nu_{aniso} - \nu_{iso} \quad [5]$$

where v_{aniso} and v_{iso} are the normal mode peak frequency position obtained by fitting respectively the anisotropic and isotropic Raman profile with the above described procedure.

NC, $(TTAGGG)_4TT$, tel-26			N	vibrational assignment
VV	HV	ISO		
1189.2±0.5	1188.8±0.8	1190±0.9	1	dT unpaired, dG
1244.0±0.3	1443.4±0.4	1244.4±0.4	2	dT NH def, CN str
1313±1	1319±1	1309±2	3	dA C8-N9, C2-N3 purine rings vibration
1336.3±0.2	1336.5±0.4	1337.6±0.3	4	dA C5-N7, N7-C8 imidazole ring vibration
1374.5±0.1	1373±0.2	1375.1±0.2	5	dT C5-CH_3 def; dG
1417.4±0.3	1417.7±0.4	1417.4±0.6	6	C2'-H_2 sc(B,Z,DNA)
1485.64±0.04	1486.6±0.05	1484.18±0.08	7	dG N7=C8 str (Hoogsteen H-bond),dA C4-N9 str, C8-H bend
1510.5±0.5	-	1503±4	8	dA ring mode
1536.1±0.4	1537±0.9	1536.6±0.7	9	dG C4-N9 str, C6=O6 str (H-bond), N1-C2
1579±0.1	1579.1±0.1	1578.8±0.3	10	dG N2-H def (H-bond)
1608.4±0.2	1610±0.4	1607.3±0.4	11	dG N1-H in-plane bend (H bond)
1656.7±0.3	1655.5±0.7	1657.3±0.6	12	dT C4=O4 str (H-bond), C5-C6 str
1685± 6	1684±5	1686±15	13	dG C6=O6 str (H-bond)

Table S1: UV Resonant Raman markers (cm^{-1}) at λ=266 for VV, HV and ISO spectra of NC aqueous solution at 25 °C.

SC, $(TTAGGG)_4TT$, tel-26			N	vibrational assignment
VV	HV	ISO		
1189.3±0.6	1188.6±0.9	1190±1	1	dT unpaired, dG
1243.6±0.3	1242.8±0.4	1244.6±0.5	2	dT NH def, CN str
1312.6±0.9	1316.8±0.8	1307±1	3	dA C8-N9, C2-N3 purine rings vibration
1337.7±0.2	1337.1±0.2	1338.7±0.2	4	dA C5-N7, N7-C8 imidazole ring vibration
1374.5±0.1	1372.5±0.2	1375.1±0.2	5	dT C5-CH_3 def; dG
1417.7±0.3	1418.6±0.5	1418.4±0.7	6	C2'-H_2 sc(B,Z,DNA)
1484.74±0.04	1485.42±0.06	1483.52±0.08	7	dG N7=C8 str (Hoogsteen H-bond),dA C4-N9 str, C8-H bend
1509.6±0.5	1509.3±0.5	1509.2±0.6	8	dA ring mode
1537.3±0.6	1535.9±0.9	1535.2±0.9	9	dG C4-N9 str, C6=O6 str (H-bond), N1-C2
1579.5±0.1	1579.6±0.1	1579.2±0.2	10	dG N2-H def (H-bond)
1607.2±0.2	1609.5±0.4	1605.7±0.3	11	dG N1-H in-plane bend (H bond)
1656.1±0.4	1655.0±0.7	1657.3±0.4	12	dT C4=O4 str (H-bond), C5-C6 str
1684.5±3.9	1685±2.5	1683±9	13	dG C6=O6 str (H-bond)

Table S2: UV Resonant Raman markers (cm^{-1}) at λ=266 for VV, HV and ISO spectra of SC aqueous solution at 25 °C.

PC, $(TTAGGG)_4TT$, tel-26			N	vibrational assignment
VV	HV	ISO		
1182.1±0.5	1180.8±0.7	1184±1	1	dT unpaired, dG
1241.6±0.3	1241.6±0.4	1243.1±0.5	2	dT NH def, CN str
1311.8±0.3	1316.3±0.4	1306.9±0.4	3	dA C8-N9, C2-N3 purine rings vibration
1335.9±0.1	1335.9±0.2	1336.4±0.1	4	dA C5-N7, N7-C8 imidazole ring vibration
1371.8±0.1	1370.4±0.2	1373.1±0.2	5	dT C5-CH_3 def; dG
1417.7±0.2	1417.5±0.3	1417.5±0.4	6	C2'-H_2 sc(B,Z,DNA)
1483.79±0.03	1484.38±0.03	1482.49±0.06	7	dG N7=C8 str (Hoogsteen H-bond),dA C4-N9 str, C8-H bend
1509±0.2	1508.5±0.5	1508.3±0.3	8	dA ring mode
1533.7±0.3	1533.9±0.5	1533.2±0.4	9	dG C4-N9 str, C6=O6 str (H-bond), N1-C2
1576.67±0.06	1576.65±0.06	1576.8±0.1	10	dG N2-H def (H-bond)
1604.6±0.1	1606.4±0.2	1602.8±0.2	11	dG N1-H in-plane bend (H bond)
1657±0.8	1656.2±0.4	1660±1	12	dT C4=O4 str (H-bond), C5-C6 str
1689±2	1687±1	1691±2.8	13	dG C6=O6 str (H-bond)

Table S3: : UV Resonant Raman markers (cm^{-1}) at λ=266 for VV, HV and ISO spectra of PC aqueous solution at 25 C°.

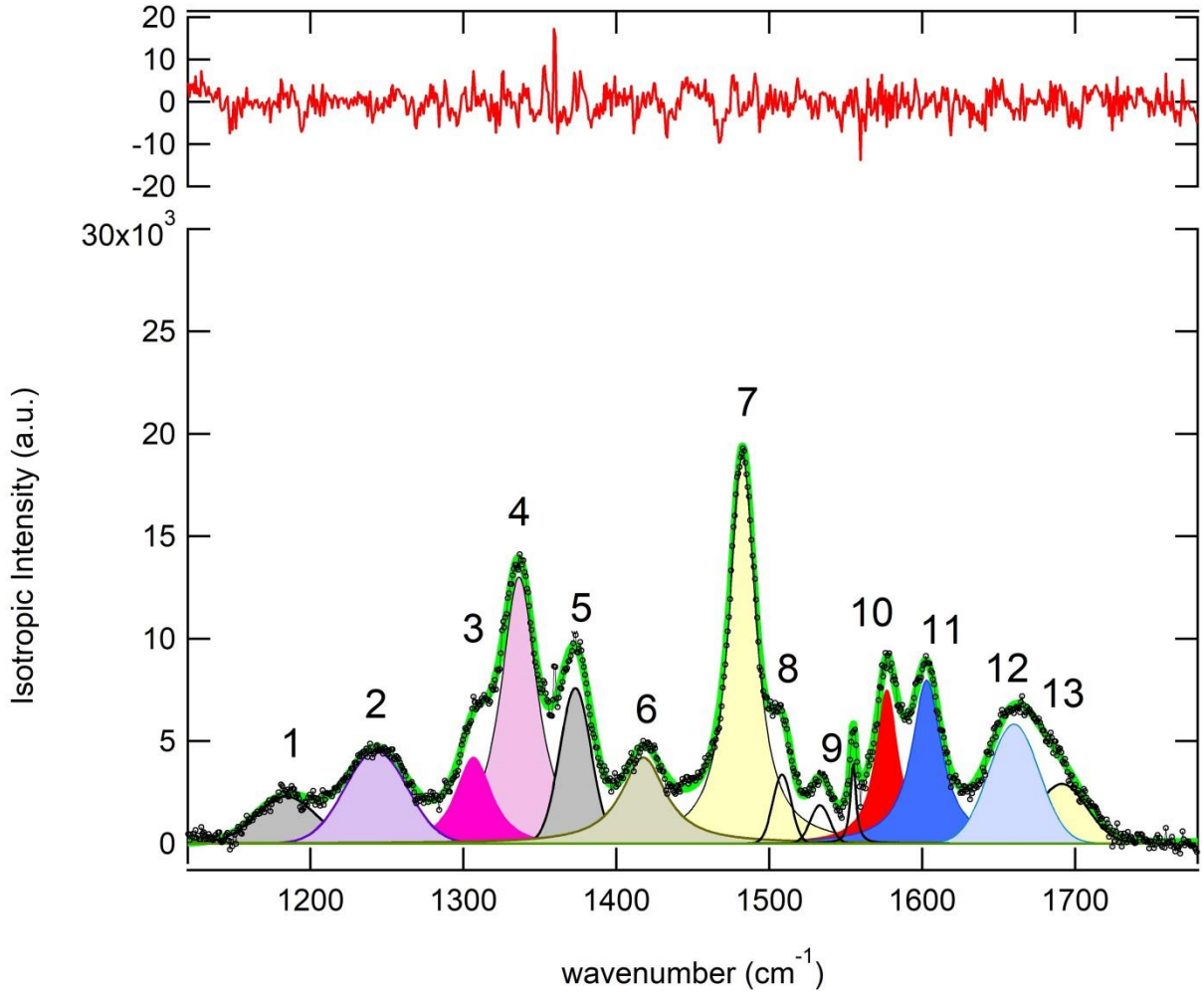

Figure S9: Green line: KAF fitting of the isotropic UV resonant Raman intensity at λ=266 nm for PC solution at T=25 °C. Numbers indicate vibrational assignment and wavenumbers for the position of the maxima, as indicated in Supplementary Table S3**.** The sharp peak between peaks 9 and 10 corresponds to the symmetric stretch of oxygen in air at 1556.4 cm^{-1}, due to position of the focal plane close to the surface of the sample.

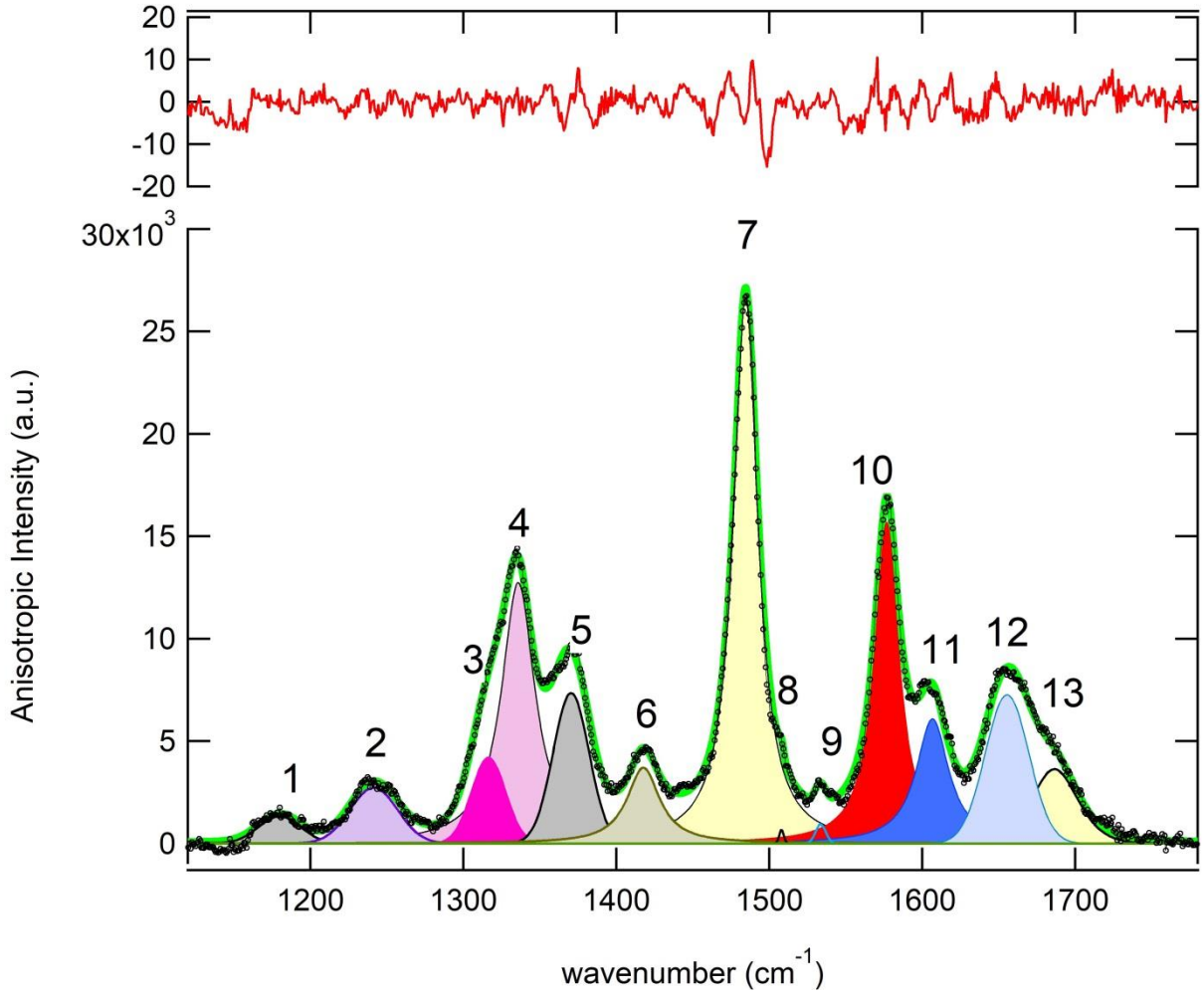

Figure S10: Green line: KAF fitting of the **an**isotropic UV resonant Raman intensity at λ=266 nm for PC at T=25 °C. Numbers indicate vibrational assignment and wavenumbers for the position of the maxima, as indicated in Supplementary Table S3**.**

Quantum chemical computations

Raman activities (S_i) were converted to relative Raman Intensities (I_i) using the following relationship derived from the theory of Raman scattering [3]:

$$I_i = \frac{A(\nu_0 - \nu_i)^4 S_i}{\nu_i\left[1-\exp(-\frac{hc\nu_i}{kT})\right]} \quad [1]$$

where ν_0 is the excitation frequency (in cm^{-1} units), ν_i is he vibrational wavenumber of the *i*th normal mode, h is the Planck constant, c is the speed of light, k is the Boltzmann constant and A is a common normalization factor for all peak intensities.

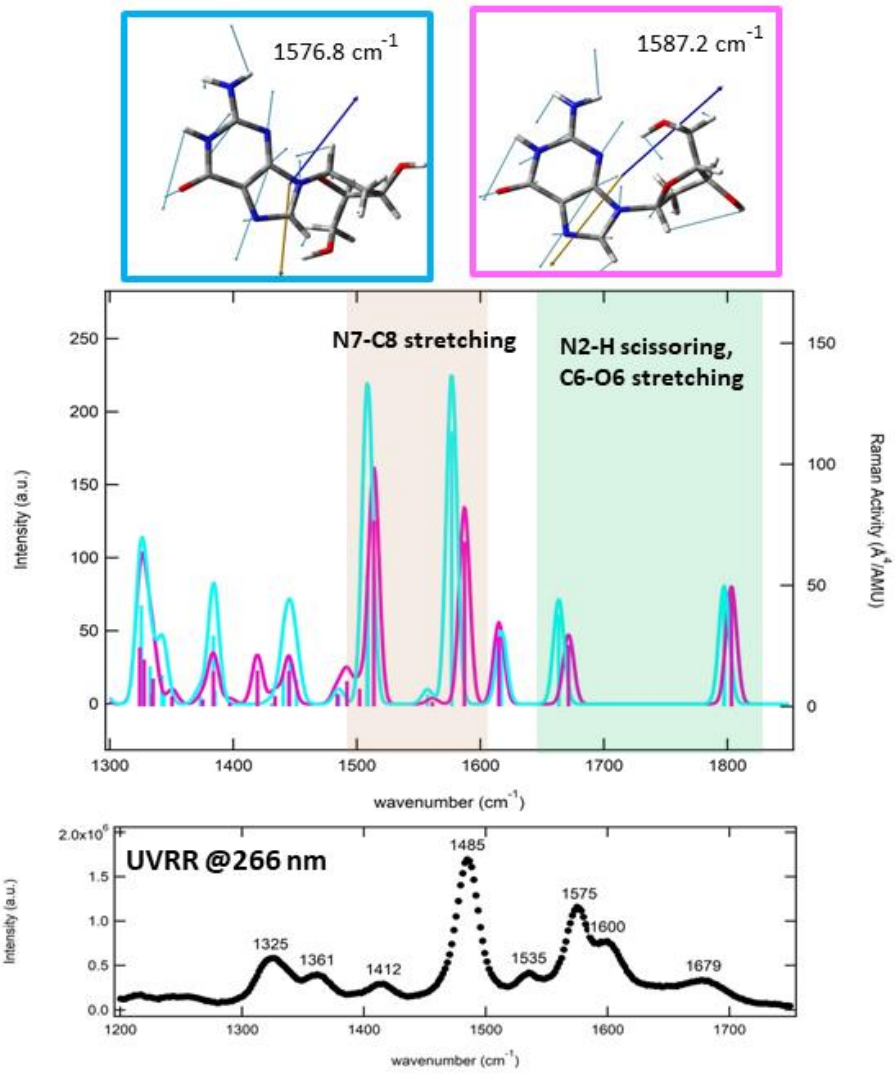

Figure S11: at the top, schematic pictures for the most intense N7-C8 stretching vibrations for deoxyguanosine molecule in the *anti*-conformation *(*cyan frame*)* and *syn*-conformation (magenta frame). Displacement vectors are indicated in green. Transition dipoles are indicated in yellow and dipole moments in blue. At the center, simulated Raman activity and Intensity profiles for the ground state molecular structure of deoxyguanosine in syn-conformation (magenta) and anti-conformations (cyan). Bars refer to Raman activity (right scale). Continuous lines refer to the intensity (left scale) obtained by convolution with a Gaussian band shape function of bandwidth (FWHM) of 5 cm^{-1}. At the bottom, experimental VV polarized UVRR spectrum for 40 μM deoxyguanosine triphosphate solution.

***anti*- conformation**			***syn*- conformation**		
Mode #	**freq (1/cm)**	**Raman Activity (A^4/AMU)**	**Mode #**	**freq (1/cm)**	**Raman Activity (A^4/AMU)**
			58	1324.3	24.5
59	1325.4	41.8	59	1327.8	19.4
60	1332.5	16.6	60		
61	1342.2	13.0	61	1335.0	11.5
64	1383.9	29.3	64	1383.9	14.6
66			66	1419.5	14.9
67	1440.5	13.3	67		
68	1445.8	21.7	68	1445.0	14.7
69	1451.4	11.2	69		
70			70	1492.1	10.5
71	1508.3	54.6	71		
72	1509.0	50.2	72	1513.8	76.7
74	**1576.8**	**113.4**	**74**	**1587.2**	**68.1**
75	1617.1	25.7	75	1615.1	28.7
76	**1663.5**	**38.1**	**76**	**1671.4**	**25.5**
77	**1797.4**	**47.1**	**77**	**1803.3**	**47.4**

Table S4: Harmonic frequencies (RA>10) for the ground state of deoxyguanosine with the sugar in *syn*- and *anti*- conformations in the range of interest. Harmonic frequencies have been calculated with the density functional theory employing B3LYP exchange-correlation function and the 6-311G(2d,2p) basis set. Vibrations which present conformational shifts are highlighted in brown (N7-C8 stretching) and green (N2-H scissoring, C6-O6 stretching)

Supplementary References:

1. L. Mariani, A. Morresi, R. S. Cataliotti and M. G. Giorgini, *The Journal of Chemical Physics*, 1996, **104**, 914-922.
2. W. G. Rothschild, *The Journal of Chemical Physics*, 1976, **65**, 455-462.
3. V. Krishnakumar, G. Keresztury, T. Sundius and R. Ramasamy, *Journal of Molecular Structure*, 2004, **702**, 9-21.