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Hydration-dependent dynamics of human telomeric oligonucleotides in the picosecond timescale: A neutron scattering study

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The dynamics of the human oligonucleotide $AG_3(T_2AG_3)_3$ has been investigated by incoherent neutron scattering in the sub-nanosecond timescale. A hydration-dependent dynamical activation of thermal fluctuations in weakly hydrated samples was found, similar to that of protein powders. The amplitudes of such thermal fluctuations were evaluated in two different exchanged wave-vector ranges, so as to single out the different contributions from intra- and inter-nucleotide dynamics. The activation energy was calculated from the temperature-dependent characteristic times of the corresponding dynamical processes. The trends of both amplitudes and activation energies support a picture where oligonucleotides possess a larger conformational flexibility than long DNA sequences. This additional flexibility, which likely results from a significant relative chain-end contribution to the average chain dynamics, could be related to the strong structural polymorphism of the investigated oligonucleotides. © *2015 AIP Publishing LLC.* [http://dx.doi.org/10.1063/1.4923213]

INTRODUCTION

The functionality of nucleic acids is intimately connected with their microscopic structure and stability (i.e., dynamics), which in turn are both known to be strongly influenced by the presence and amount of hydration water.

The dry state of DNA is devoid of base-stacking and has no secondary structure while, as the hydration level is increased, A-DNA and B-DNA forms are progressively obtained.[1] Changes of the hydration shell may alter the DNA shape in a sequence-dependent manner,[2] thus affecting critical biochemical processes such as ligand recognition. In particular, this is the case of transitions from canonical to non-canonical DNA structures, which can be specific and important signals in nucleic acid-nucleic acid, nucleic acid-protein, and nucleic acid-drug interactions.[3] In recent years, there has been a growing interest in studying the structural polymorphism of guanine-rich oligonucleotide sequences that can form four-stranded structures called G-quadruplexes.[4] Guanine-rich regions are frequently present in genomes, especially in gene promoters, and their secondary structures may have important biological functions relevant to cancer treatment.[4] Molecular crowding can strongly affect the stability and even induce the formation of these secondary structures.[5] It has been shown, for example, that when water activity decreases and hydration level is poor, DNA structures containing Hoogsteen base pairs are more stable than those with Watson-Crick base pairs.[2] Indeed, studies of the oligonucleotide features in molecular crowding conditions are crucial in the quest to understand physiology and metabolism of cells *in vivo* (see, for example, Ref. 6).

The conformational properties of the different DNA regions are also strictly related to fast thermal fluctuations, as long-range mechanical properties of DNA have their origin in the picosecond timescale dynamics of molecule-sized segments.[7] Experimental investigations have shown that this picosecond timescale dynamics has a diffusive character more typical of fluids, rather than the purely vibrational character of crystals.[8,9] In addition, it has been shown by neutron scattering measurements that the onset of DNA fast thermal fluctuations is ruled by hydration water[10–12] and can be modulated by changing the molecular environment around the biomolecular surface.[13]

In this framework, we have performed an incoherent neutron scattering study of the $AG_3(T_2AG_3)_3$ oligonucleotide, the human telomeric repeat that was shown to form intramolecular G-quadruplexes[14] and whose stability properties in the context of molecular crowding have been recently investigated (see, for example, Ref. 5). By fixed-window elastic incoherent neutron scattering (EINS) measurements, we could estimate the amplitude of the oligonucleotide thermal fluctuations when

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it is not yet folded in the G-quadruplex structure, over a wide temperature range. Quasielastic neutron scattering (QENS) was then exploited to reveal the characteristic timescale of such thermal fluctuations. The picosecond dynamics of the $AG_3(T_2AG_3)_3$ oligonucleotide was measured at low water content, i.e., in conditions of heavy molecular crowding, so that also the signal from the solvent itself can be neglected compared to that of the biomolecule. Under these conditions, the investigated system possibly resembles the condensed phase of DNA in chromatin, where, due to the high packing of DNA and its binding to histones, the DNA intermolecular interactions are enhanced and the global diffusion is significantly limited.

In detail, we found out that oligonucleotides undergo a hydration- and temperature-dependent dynamical activation, which is similar to that of hydrated protein powders[15] but shows the signature of a specific interplay with the surrounding water molecules. From a comparison with further EINS and QENS measurements on complete DNA samples from salmon testes, we could highlight a much larger molecular flexibility of oligonucleotides. This behaviour is probably due to the significant relative chain-end contribution to the average DNA dynamics in short oligonucleotides at low hydration conditions, surprisingly resembling what happens in dilute solutions.

MATERIALS AND METHODS

Sample preparation

Lyophilized DNA oligonucleotide sequence $AG_3(T_2AG_3)_3$ was purchased from Eurogentec (Seraing, Belgium) with a nominal purity level of 98.6%. The oligonucleotides were dialyzed against 1 l of a 150 mM NaCl aqueous solution for 12 h, so as to leave only sodium counterions. They were then dissolved in heavy water at a concentration of about 0.05 mg/ml and left at room temperature for 1 day to properly substitute all the exchangeable hydrogen atoms with deuterium. Such dilute solution was freeze-dried and further dehydrated under vacuum in presence of P_2O_5, in order to obtain the lowest achievable water content. The dry powder was subsequently hydrated at 75% relative humidity, using NaCl saturated solutions of D_2O, until a hydration level $h = 0.7$ (h = grams water/grams DNA oligonucleotide) was achieved. Lower hydration degrees ($h = 0.55$, 0.4, 0.2, and 0) were obtained by subsequently dehydrating the sample under vacuum.

DNA sodium salt from salmon testes was purchased from Sigma-Aldrich (St. Louis, MO) and used with no further purification. The sample was prepared with same procedure as above to reach a hydration level $h = 0.55$.

All samples were sealed in standard 0.2 mm thick aluminium cells, which were used for temperature-dependent neutron measurements. The hydration level was monitored by weighing the samples before and after each measurement and was observed to remain constant.

Incoherent neutron scattering concepts

Neutron scattering spectroscopy is widely used to directly probe fast internal motions of biomolecules. In a neutron scattering experiment, the molecular motions are investigated by measuring the dynamic structure factor $S(Q,E)$, which represents the probability of an incident neutron to be scattered by the sample with an energy transfer E and a momentum transfer $\hbar Q$,[16] where Q is the wave-vector transfer. The dynamic structure factor includes both coherent and incoherent contributions that arise from collective and single-particle atomic motions, respectively.

Hydrogen atoms are characterized by a very large incoherent cross section (σ_{inc} = 79.90 b vs. σ_{coh} = 1.76 b), which is by far larger than the coherent and incoherent cross sections of deuterium and of any other element.[16] Because deuterated water was used in our samples, the most significant contribution to the detected signal is incoherent and comes from the oligonucleotide or salmon DNA non-exchangeable hydrogen atoms. These atoms are copiously and uniformly distributed throughout the nucleic acids macromolecules, allowing a complete sampling of both their vibrational motions and their confined diffusive dynamics arising from rotational and torsional motions of sugar rings, nucleobases, and phosphate molecular groups. In addition, because all the samples are isotropic from the molecular to the macroscopic scale, the measured intensity will depend only on the modulus of the exchanged momentum vector. In the incoherent approximation,[16] the dynamic structure factor can be written as

$$S(Q,E) = \left\{ e^{-\langle u^2 \rangle Q^2} \left[A_0(Q)\,\delta(E) + (1 - A_0(Q))\,L(Q,E) \right] \right\} \otimes R(Q,E), \quad (1)$$

where all the terms are convoluted with the experimental resolution function $R(Q,E)$. Apart from the so-called Debye-Waller factor, which describes the Gaussian intensity decrease due to quasi-harmonic vibrational atomic mean square displacements (MSD) $\langle u^2 \rangle$, the dynamic structure factor in Eq. (1) is split into the sum of two components.

The first term represents the elastic response of the system, whose energy dependence is given by a Dirac delta function, whereas the modulation as a function of Q is provided by the elastic incoherent scattering factor $A_0(Q)$.[16] This quantity represents the space-Fourier transform of the scatterers' distribution, taken at infinite time and averaged over all the possible initial positions. As by definition $A_0(Q)$ is the ratio between the elastic intensity and the total scattered intensity, for small Q values, $A_0(Q)$ tends to 1. This first term of Eq. (1) is usually measured by EINS experiments.

The second term, appearing in the spectra as an energy broadening of the elastic peak, accounts for the relaxational

motions sampled by the hydrogen atoms. In our case, the product of the quasielastic incoherent scattering factor $(1 - A_0(Q))$ and a single Lorentzian function $L(Q,E) = \frac{1}{\pi}\frac{\Gamma(Q)}{E^2+\Gamma(Q)^2}$ plus a flat background satisfactorily reproduces all the experimental features of the data. Such a quasielastic component is characterised by its half-width at half maximum Γ, which in principle can be Q-dependent and is related to motions with characteristic time $\tau = \hbar/\Gamma$.[16] This second term is measured by QENS experiments.

As a remark, in Eq. (1), no purely inelastic term appears, because in the low-energy transfer range explored in the present experiments, the vibrational contribution can be safely neglected with respect to the elastic and quasielastic terms.

If the scattering intensity is recorded only within a narrow energy interval centered at $E \sim 0$ and comparable in width to the experimental energy resolution, also the quasielastic term can be neglected and Eq. (1) takes the simpler form

$$S(Q,E \sim 0) = \left[e^{-\langle u^2\rangle Q^2} A_0(Q)\right] \otimes R(Q,E). \qquad (2)$$

Neutron experiments

EINS measurements were performed on the high-resolution, wide-momentum-transfer backscattering spectrometer IN13 (ILL, Grenoble, France).[17] With an incident wavelength of 2.23 Å, an energy resolution with a full-width at half-maximum (FWHM) of about 8 μeV and an average wave-vector transfer resolution of about 0.2 Å^{-1} were achieved in the Q range 0.5–4.48 Å^{-1}. $AG_3(T_2AG_3)_3$ oligonucleotides at $h = 0$, 0.2, 0.4, and 0.7 were investigated from 20 to 300 K. The acquired data were treated in order to correct for incident flux, cell scattering, sample self-shielding, and detector efficiency. The elastic intensity of each sample relative to a given temperature was normalized with respect to the data collected at 20 K.

A QENS experiment was performed on the backscattering spectrometer SPHERES at FRM II (Garching, Germany),[18] over the rather wide energy range from −25 to 25 μeV and at an energy resolution of 0.65 μeV (FWHM). Such a resolution function makes accessible only motions faster than about 2 ns. The investigated Q range was from 0.6 to 1.76 Å^{-1}. Spectra were collected at temperatures T = 250, 270, 285, and 300 K for the $AG_3(T_2AG_3)_3$ oligonucleotides at h = 0.55, and at T = 250 and 300 K for salmon sperm DNA at the same hydration level. An empty cell measurement was subtracted from the samples' spectra that were then corrected for the angular dependence of the detector efficiency by normalization to a vanadium standard. The energy resolution function was evaluated through a measurement of the samples at 3 K.

In both IN13 and SPHERES measurements, an average transmission of 93% was obtained. Multiple scattering contributions were neglected.

RESULTS AND DISCUSSION

In Fig. 1, we plot the normalized elastic intensities as a function of temperature for oligonucleotides $AG_3(T_2AG_3)_3$ at h = 0, 0.2, 0.4, and 0.7, integrated over the low momentum region from 0.5 to 1.86 Å^{-1}, corresponding to an average wave-vector transfer Q_{av} of 1.2 Å^{-1}. Within the incoherent approximation, any departure from unity of the normalized elastic intensity gives a qualitative and model-independent indication of the oligonucleotides mobility. In fact, the most striking feature of the data in Fig. 1 is a progressive drop from unity on increasing temperature. Despite it occurs for all hydration levels, the intensity drop becomes abruptly more marked for the most hydrated samples and at temperatures around 200-240 K. Such a phenomenon, observed in many hydrated biological systems such as proteins, DNA, and tRNA,[11,15,19,20] is known as *dynamical transition* and has been extensively debated in the literature.[19,21–27] As concerns DNA oligonucleotides, this is the first experimental evidence of such a transition, which can be related both to the activation of segmental motions of the DNA chain (inter-nucleotide dynamics) and to internal fluctuations of phosphate groups, sugars, and nucleobases (intra-nucleotide dynamics). The dynamical transition is also known to generally show a strong dependence on the environment surrounding the biomolecules.[15,20,28–30] It has been suggested to possibly initiate biological functionality in proteins,[22,31] where it takes place in the same temperature range. Since the dynamical transition does not occur in dry biomolecules, it has been inferred that thermal fluctuations are enhanced by the lubricant action of the solvent via the translational displacements of water molecules. In fact, MD simulations have shown that in the case of proteins the inhibition of the solvent translational mobility has an effect similar to dehydration on the dynamical transition.[32–35]

The second remarkable feature emerging from Fig. 1 is a clear dependence of the normalized elastic intensity on the hydration level. Distinctively, from room temperature down to about 250 K, the elastic intensity of the most hydrated

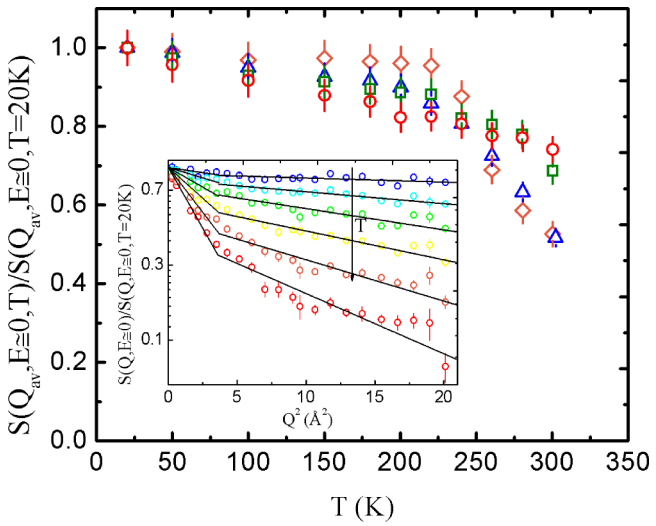

FIG. 1. Normalized incoherent elastic intensities of oligonucleotides as a function of temperature, integrated over the momentum transfer range corresponding to an average wave-vector transfer Q_{av} of 1.2 Å^{-1}, at h = 0 (red circles), h = 0.2 (green squares), h = 0.4 (blue triangles), and h = 0.7 (orange diamonds). Inset—Normalized incoherent elastic intensities versus Q^2 of the sample hydrated at h = 0.4 and at selected temperatures: 100, 200, 240, 260, 280, and 300 K from top to bottom. Lines: fits to the data.

samples ($h = 0.4$ and $h = 0.7$) is lower than that of the dry and $h = 0.2$ samples. In addition, the hydration dependence of the elastic intensity appears to be rather nonlinear, as it presents a sudden intensity drop—particularly well visible at the highest temperatures—in passing from $h = 0.2$ to $h = 0.4$. Interestingly, such a neat jump occurs at a hydration level corresponding to the full hydration of phosphate groups along the DNA chain (6-7 water molecules per PO_4 group, i.e., $h \sim 0.3$).[36,37] Below this threshold, all water molecules are tightly bound to phosphate groups and the mobility of the oligonucleotide-water system is reduced. An analogous behaviour has been found for oligopeptides, once the relevant hydrophilic sites are saturated.[38] It is also worth of notice that a constrained dynamics is suggested for the most hydrated sample in the low-temperature region (from 150 to about 220 K), probably due to the confining effect of the glass-like layer of water at the biomolecule surface. A similar effect has been observed also in peptides and proteins hydrated powders.[39,40]

Further insights into the nature of the observed intensity drop can be gained from its Q^2-dependence, which is plotted in the inset of Fig. 1 for the sample hydrated at $h = 0.4$ and at selected temperatures (namely, 100, 200, 240, 260, 280, and 300 K). On qualitative grounds, the samples at the other investigated hydration levels exhibit similar trends (data not shown). In the logarithmic vertical scale of the plot, a Gaussian behaviour would appear as a straight line. In fact, we observe a clearly non-Gaussian trend of $S(Q, E \sim 0)$, which starts above 100 K and becomes more and more pronounced as the temperature increases. The origin of such a non-Gaussian trend has to be ascribed to the onset of anharmonic contributions.

In the literature, two kinds of anharmonic motions are known to usually take place in hydrated biological systems. The first contribution is observed at temperatures right above 100 K and has generally been attributed to the rotational motion of methyl groups.[41–43] Indeed, it was found in lysozyme and other proteins, while it is absent in tRNA where CH_3 groups are missing.[44,45] In the human telomeric repeat, where roughly 10% of the nonexchangeable hydrogen atoms belong to methyl groups located in thymine nucleobases, we expect such a feature to be weakly present. The second anharmonic contribution becomes active around 200-240 K and yields the dynamical transition discussed above.

A deeper characterization of oligonucleotides mobility can be achieved by a more quantitative analysis of the total mean square displacements. To this end, we divided the experimental Q-range into a low- and a high-Q region, where we separately examined the Q-dependence of the elastic intensity loss, in analogy to what done for proteins and their hydration water.[46,47] More precisely, the low-Q region we chose spans from 0.5 to 1.86 $Å^{-1}$ and coincides with the interval from which the integrated intensities in the main panel of Fig. 1 were obtained. Such a wave-vector range corresponds to an average investigated length scale of about 5.5 Å, thus providing information on large-scale inter-nucleotides thermal motions. The complementary high-Q region goes from 2 to 4.48 $Å^{-1}$, corresponding to an average investigated length scale of about 2 Å, and is therefore dominated by local intra-nucleotide atomic movements. In each of the two Q-intervals, temperature-dependent MSD amplitudes were calculated from the slopes of two straight lines fitted to $\ln(S(Q,0,T)/S(Q,0,20\,\mathrm{K}))$ vs. Q^2. The neat slope difference, appearing in the inset of Fig. 1 between the two Q-ranges, witnesses a clear departure from the Gaussian approximation.

In panels (a) and (b) of Fig. 2, we show the resulting MSD of the oligonucleotides as a function of T, in the high- and low-Q regions, respectively. In both ranges, a linear trend for $\langle u^2 \rangle$ is found at low temperatures, where the main contribution is purely vibrational. The sudden increase of $\langle u^2 \rangle$ at high temperatures is instead brought about by the activation of those anharmonic motions that give rise to the dynamical transition discussed above. Similarly to the trend found for hydrated lysozyme powders,[15,41] the dynamical transition qualitatively depends on the hydration level of oligonucleotides, as in both Q-regions it progressively shifts from ~240 K to ~180 K on increasing the water content. The presence of a dynamical transition in oligonucleotides confirms the picture that the lack of a well-defined three-dimensional structure does not prevent such phenomenon from taking place. Indeed, the dynamical transition has been also observed in single-stranded DNA,[11] in hydrated oligopeptides[38,39] and polypeptides,[48] in hydrated amino acids mixtures,[49] and in intrinsically disordered proteins.[35]

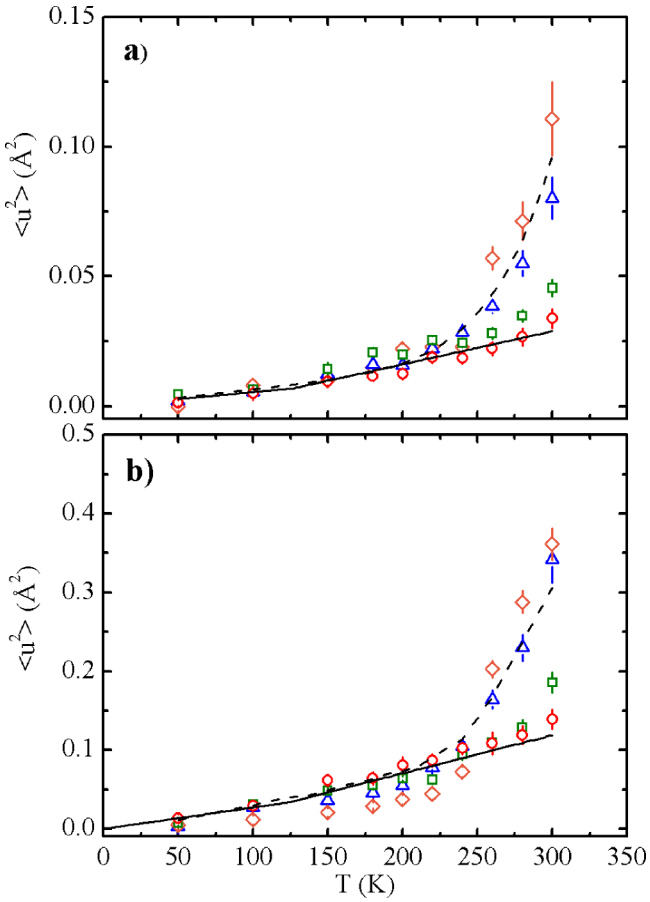

FIG. 2. Total mean square displacements versus T for oligonucleotides at $h = 0$ (red circles), $h = 0.2$ (green squares), $h = 0.4$ (blue triangles), $h = 0.7$ (orange diamonds), and for salmon sperm DNA at $h = 0$ (black full line) and $h = 0.55$ (black dashed line), calculated, respectively, in the high (panel (a)) and low (panel (b)) Q-ranges.

It is worth of further notice that, as observed for the hydration dependence of the integrated intensities in Fig. 1, below 220 K the samples at $h = 0.4$ and 0.7 show a reduced mobility with respect to the less hydrated ones. Nevertheless, the effect is present only at low Q values, thus suggesting that the hypothesised constraining action of the water layers around the biomolecular surface affects the inter-nucleotide dynamics only.

In order to obtain a more general picture, we compare the present results on the human oligonucleotide sequence $AG_3(T_2AG_3)_3$ with earlier EINS measurements on 3000-bases-long DNA from salmon sperm[11] hydrated at $h = 0$ and $h = 0.55$. To this end, we re-analyzed the old elastic data within the same scheme proposed here, i.e., by separately deriving the MSD in the two Q-regions chosen above.

In the high-Q region, the MSD values of salmon sperm DNA at $h = 0$ superimpose to those of dry oligonucleotides, while at $h = 0.55$ they lay reasonably between those of oligonucleotides at $h = 0.4$ and $h = 0.7$ (see Fig. 2(a)). This finding suggests that the intra-nucleotide thermal motions in DNA poly- and oligo-nucleotides are neither significantly affected by conformational properties such as high-order structure nor by differences in base sequence or sequence length.

On the contrary, at low Qs, a larger mobility of the oligonucleotides compared to that of salmon sperm DNA polynucleotides observed in Fig. 2(b). Referring indeed to the hydrated samples, the MSD values of salmon sperm DNA at $h = 0.55$, which corresponds approximately to the fraction of unfreezable DNA hydration water,[50] strongly resemble that of oligonucleotides at $h = 0.4$. The fact that a higher hydration level is necessary to confer DNA with the same mobility as oligonucleotides witnesses a larger mobility of the latter. It is interesting to further note that, at room temperature, the MSD of DNA end up laying between those of oligonucleotides at $h = 0.2$ and $h = 0.4$. As mentioned above, such a hydration range for the oligonucleotides and DNA corresponds to the condition of fully hydrated phosphate groups, i.e., $h \sim 0.3$.[36,37] This might suggest that, independently of the hydration level, similar mobility of the different biomolecules is achieved when the relevant hydrophilic groups are fully hydrated.

As regards the dehydrated samples, above 250 K the MSD calculated at low Q values are slightly but systematically larger in dry oligonucleotides than in dry DNA, thus reinforcing what found for the corresponding hydrated systems. This effect cannot be ascribed to the different contributions of anharmonic motions of methyl groups, as their fraction is about the same in both systems.

The emerging picture, indicating an overall higher flexibility of oligonucleotides with respect to salmon DNA, could be related to intrinsic structural properties, e.g., the length of the DNA sequence, as we will discuss in more detail below.

To get access to the timescale of the involved thermal motions, we also performed a high-resolution QENS experiment on $AG_3(T_2AG_3)_3$ oligonucleotides at $h = 0.55$. In addition, salmon sperm DNA at $h = 0.55$—where all water molecules are supposed to interact with the biomolecule—was investigated to serve as a reference.

Fig. 3 shows the spectra of both oligonucleotides and salmon sperm DNA (inset) at different temperatures. As the measured dynamic structure factors $S(Q,E)$ appeared clearly Q-independent (not shown), the spectra were summed over all the Q-range to improve the statistical significance of the data. By fitting the data to Eq. (1), we obtained the half-widths at half-maximum (Γ) of the quasielastic Lorentzian component, whose temperature dependence is plotted in Fig. 4 for the oligonucleotide sample. The inverse of these quantities provides the timescale of the thermal fluctuations: $\tau = \hbar/\Gamma$. At the investigated temperatures, the widths of QENS spectra in oligonucleotides are larger than in salmon sperm DNA, and the corresponding characteristic timescales are therefore shorter. In particular, a characteristic time τ of about 190 ± 7 ps ($\Gamma = 3.49 \pm 0.13$ μeV) is found for oligonucleotides at room temperature, while for salmon DNA it increases to about

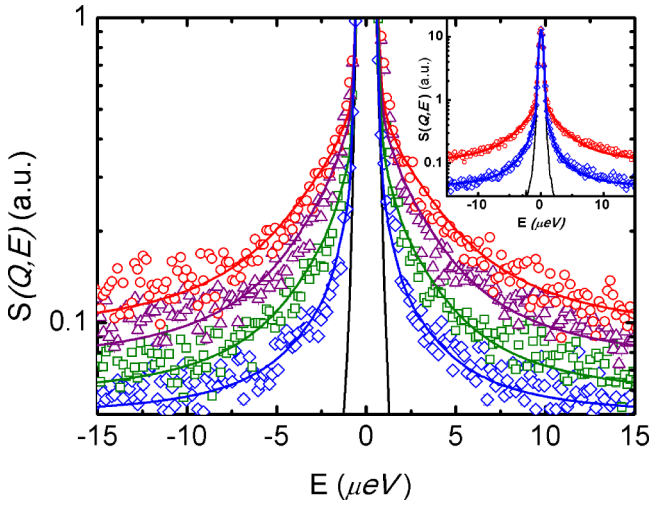

FIG. 3. Quasielastic dynamic structure factor $S(Q,E)$ of oligonucleotides hydrated at $h = 0.55$, and at temperatures of 250 (blue diamonds), 270 (green squares), 285 (purple triangles), and 300 K (red circles). Inset: Quasielastic dynamic structure factor $S(Q,E)$ of salmon sperm DNA hydrated at $h = 0.55$, at temperatures of 250 (blue diamonds) and 300 K (red circles). Black line: instrumental resolution function. Color lines: fits to the data.

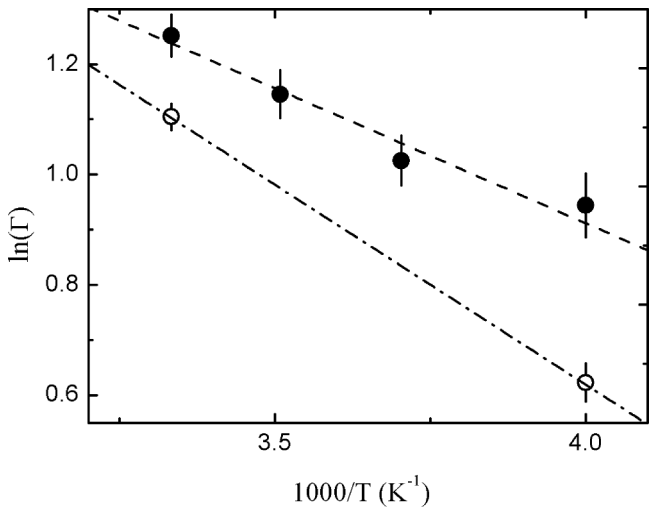

FIG. 4. Temperature dependence of the half-width at half-maximum Γ obtained from fits of a single-Lorentzian function to the Q-averaged QENS spectra of oligonucleotides (full circles) and salmon sperm DNA (empty circles), both hydrated at $h = 0.55$. Black dashed lines: fits to the experimental data with an Arrhenius dependence.

220 ± 5 ps (Γ = 3.01 ± 0.07 μeV) in the same conditions. Such a difference becomes even more accentuated at 250 K, where oligonucleotides display a characteristic time of about 255 ± 15 ps (Γ = 2.57 ± 0.15 μeV) and salmon DNA is characterized by a τ of about 350 ± 12 ps (Γ = 1.86 ± 0.07 μeV). These results imply that DNA oligonucleotides exhibit a higher flexibility not only because they explore a larger conformational space (larger $\langle u^2 \rangle$) but also because they do it in shorter times. In addition, since in both samples the Γ-values exhibit no significant Q-dependence, the corresponding motions are spatially localized, as similarly observed in lysozyme hydrated powders, tRNA, or hydrated β-lactoglobulin powders.[20,51]

As listed in Table I, the characteristic time of DNA found in this work is in good agreement with what observed previously by Khodadadi *et al.* with a similar instrumental energy resolution.[52] In detail, at room temperature, the relaxation times of both oligonucleotides and salmon sperm DNA are longer than for tRNA, protein powders at similar hydrations, or protein solutions at room temperature.[20,53–55]

This behaviour suggests that, in the picosecond range, the motional characteristic time is rather peculiar of the specific biosystem class, amounting about to 100 ps for proteins, 100 ps as well for cells[20,53–57]—whose biological components mostly consist of proteins—150 ps for tRNA,[20] and 200 ps for DNA.[52] This difference may originate from the fact that the number of water molecules strongly coordinated with polar groups is definitely larger for DNA than for proteins, due to the higher phosphate-groups content in the former. Since the dynamical activation of biomolecules mainly arises from diffusive translational motions of water molecules that are less tightly bound to the biomolecule surface,[32–34] at a given hydration level such a dynamical activation will be less significant in DNA than in proteins.

More quantitatively, the observed temperature dependence of Γ is well described by a single Arrhenius function $e^{\frac{-\Delta E}{RT}}$, with ΔE = 4.0 ± 0.5 kJ/mol for oligonucleotides. For salmon sperm DNA, the calculated activation energy ΔE = 6.0 ± 0.5 kJ/mol is larger than for oligonucleotides, consistently with the picture emerging from the MSD behaviour.

TABLE I. Characteristic times in the sub-nanosecond range of various biological systems at room temperature, together with the corresponding instrumental energy resolution.

Sample (T = 290–300 K)	τ (ps)	Energy resolution (μeV)
$AG_3(T_2AG_3)_3$ (h = 0.55)[a]	190 ± 7	0.65
DNA (h = 0.55)[a]	220 ± 5	0.65
DNA (h = 0.65)[52]	200	0.8
tRNA (h = 0.5)[20]	140 ± 20	0.8
Lysozyme (h = 0.5)[20]	95 ± 9	0.8
Crambin (h = 0.67)[53]	100	0.9
Myoglobin in solution (200 mg/ml)[54]	66	0.8
Bacteriorhodopsin in purple membranes (h = 0.56)[55]	120	1.8
Haemoglobin in red blood cells[56]	110	0.9
E.coli cells[57]	90	0.9

[a]Present work.

Such values result for the most part from the activation energy of weak hydrogen bonds around hydrophilic sites of nucleotides, since only a small fraction of DNA hydrogen atoms belong to hydrophobic groups, such as CH_3. For instance, MD simulations and experiments on polypeptides in the presence of water reported ΔE values for weak hydrogen bonds between 2 and 8 kJ/mol.[58] As a remark, the activation energy of hydrogen bonds between water molecules is known to be of the order of 8 kJ/mol, emphasizing again the mutual interaction with the dynamics of the solvent, whereas that of backbone methyl groups in model peptides was found to be about 5.5 kJ/mol.[39]

Globally, our results indicate a large degree of flexibility of oligonucleotides. The comparison with a similar biosystem, such as salmon sperm DNA, is helpful to gain insights into the molecular basis of this characteristic mobility. As a first remark in this sense, the higher flexibility of $AG_3(T_2AG_3)_3$ oligonucleotides should not be ascribed to their specific tertiary structure, since the MSD of double- and single-stranded salmon sperm DNA were shown to be actually coincident at the same hydration level and temperature.[11] The latter indicates instead that, as far as picosecond thermal fluctuations are concerned, the effect of hydrogen bonds between complementary bases is equivalent to that between a single base and bound water molecules. The dynamical difference between oligonucleotides and salmon sperm DNA found at low Q values cannot be attributed to distinct environmental factors either, such as hydration level or nature and concentration of counterions, because these are about the same in all the investigated samples (about one sodium atom per phosphate group). The larger flexibility of oligonucleotides has therefore to be ascribed to other—intrinsic—properties, such as sequence composition or chain length. Actually, the problem of possible variations of DNA rigidity arising from different base sequences is still highly debated, although several research groups have attempted various experimental and theoretical approaches.[59,60] As a remark, Brauns and coworkers did not observe a sequence dependence of the localized dynamics in B-DNA oligonucleotides within the time range from 40 ns to 40 ps by time-resolved Stokes shift measurements.[61] Some dynamical features of nucleic acids, like the hydrodynamic self-diffusion coefficient and the nanosecond rotation time,[62,63] have already been shown to depend on chain length. Fluorescence spectroscopy studies have reported a chain-length dependent flexibility in the time-scale of seconds.[64] On these grounds, the increased mobility we observed for oligonucleotides could also be attributed to a chain-length effect. In fact, recent MD simulations of DNA dilute solutions have shown that the terminal DNA residues display a higher mobility with respect to the central part of the chain,[65] as it happens for linear inorganic polymers.[66]

Under the hypothesis that the larger mobility of terminal nucleotides is independent of the chain length, their contribution to the average dynamics of the whole chain should increase as the length gets smaller. We can then tentatively describe the thermal fluctuations of a DNA sequence as if they resulted from two distinct classes of nucleotides belonging to the internal section (i.e., the backbone) and the terminal parts of the chain, with the corresponding MSD given by $\langle u_{bb}^2 \rangle$

and $\langle u^2_{ext}\rangle$, respectively. In this picture, the measured mean square displacements $\langle u^2\rangle$ at low Qs, which reflect large-scale inter-nucleotide motions, satisfy the following relationship:

$$\langle u^2\rangle = \frac{(N-2n)\langle u^2_{bb}\rangle + 2n\langle u^2_{ext}\rangle}{N}, \quad (3)$$

where N is the total number of nucleotides in the chain and $2n$ is the number of nucleotides that belong to the two chain-ends and whose relaxational times enter the experimental window of the spectrometer. From widely used polymer physics models, the ratio of chain-end to whole-chain dynamics is commonly estimated to decay as $1/\sqrt{N}$,[66] due to the stochastic nature of chain modes in a confined space. When N is very large, as in the case of salmon sperm DNA, the second term in the right-hand side of Eq. (3) is negligible with respect to the first one. Thus, defining $q = 2n/N$, we can rewrite the above relation as

$$\langle u^2\rangle_{DNA} \cong \langle u^2_{bb}\rangle_{DNA} \quad (4a)$$

for DNA, and

$$\langle u^2\rangle_{oligo} = (1-q_{oligo})\langle u^2_{bb}\rangle_{oligo} + q_{oligo}\langle u^2_{ext}\rangle_{oligo} \quad (4b)$$

for oligonucleotides.

The contribution $q_{oligo}\langle u^2_{ext}\rangle_{oligo}$ of the extremal parts of the chain starts to be significant above the dynamical transition temperature, that is when chain-end fluctuations progressively enter the time window of the spectrometer on increasing T. As the number of nucleotides belonging to chain-ends does not change with temperature, q_{oligo} has been fixed to 0.09, which corresponds to about 2 extremal nucleotides over the 22 composing the investigated human telomeric sequence. In turn, above the dynamical transition around 250 K, we expect the amplitudes of their corresponding motions $\langle u^2_{ext}\rangle_{oligo}$ to be significantly temperature-dependent, since they are reasonably activated by conformational dynamics and their characteristic times gradually come out of the instrument energy resolution. We also hypothesize that, at given hydration degree, $\langle u^2_{bb}\rangle_{oligo}$ equals $\langle u^2_{bb}\rangle_{DNA}$, i.e., the backbone dynamical contribution is independent of chain length. Further, as the ratio q_{DNA}/q_{oligo} between chain-end contributions in polynucleotides and in oligonucleotides scales like $\sqrt{(N_{oligo}/N_{DNA})} = 1/12$,[66] from the fixed value of q_{oligo} we can estimate that at room temperature, q_{DNA} is less than 1%, thus supporting the consistency of Eq. (4a) as well.

With the above assumptions, in the low-Q region and at high temperatures ($T > 240$ K), we first interpolated at $h = 0.55$ the oligonucleotides experimental $\langle u^2\rangle$, to which then we applied Eqs. (4a) and (4b), thus obtaining $\langle u^2_{ext}\rangle_{oligo}$ as a function of temperature and for $h = 0.55$. At 300 K, such MSD of chain-end nucleotides resolved by the IN13 spectrometer result to be 0.8 ± 0.1 Å^2. This implies that the two extremal nucleotides undergo motions with amplitudes approximately 2.3 times larger than the average oligonucleotide. Further, by applying the same simple model to the high-Q region, we find that $\langle u^2\rangle_{oligo} \cong \langle u^2_{bb}\rangle_{oligo} \cong \langle u^2_{ext}\rangle_{oligo} = 0.096$ Å^2 at room temperature, thus showing that the dynamical differences between the central part of the DNA chain and its ends are relevant only when the inter-nucleotide dynamics is considered.

In summary, we reported experimental evidence of a hydration-dependent dynamical transition in human oligonucleotides $AG_3(T_2AG_3)_3$, which we observed at the low-hydration conditions ($h = 0.2-0.7$) that mimic a crowded cellular environment. The corresponding motions have been further investigated by QENS and display characteristic times between 190 and 255 ps, at temperatures between 300 and 250 K. Based on a quantitative analysis of the measured mean square displacements and of the corresponding activation energies, we ascribe the observed large mobility of oligonucleotides to a chain-length effect, similarly to existing results for DNA dilute solutions.

As a final remark, we can suggest that the large thermal fluctuations of hydrated oligonucleotides above the dynamical transition temperatures may have a role in promoting the interactions with small molecules such as drugs, since dynamical processes on the picosecond timescale are critical at the early stage of recognition processes. On the other hand, on the basis of the present findings, we can also speculate that the higher polymorphic degree of oligonucleotides compared to long DNA sequences may be due to their increased flexibility. In fact, in the past, it has been suggested that a strong correlation exists between electrophoretic mobility and size-related parameters of oligonucleotides, such as end-to-end distance obtained from MD simulations.[67] Even though our results refer to dynamical properties in the sub-nanosecond timescale, they can be relevant for conformational changes occurring at longer timescale in a similar way as it was proposed for picosecond dynamics triggering the micro- and milli-second functional conformational changes of proteins.[68]

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