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Terahertz Dynamics in Human Cells and Their Chromatin

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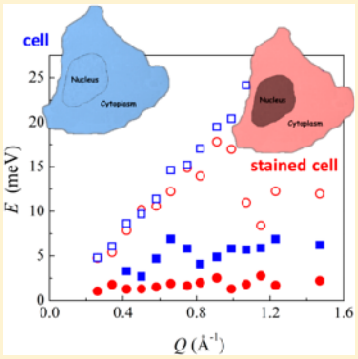
Supporting Information

ABSTRACT: The terahertz dynamics of human cells of the U937 line and their chromatin has been investigated by high-resolution inelastic X-ray scattering. To highlight its dynamical features *in situ*, nuclear DNA has been stained by uranyl-acetate salt. The general behavior of the collective dynamics of the whole cell is quite similar to that of bulk water, with a nearly wavevector-independent branch located at about 5 meV and a propagating mode with a linear trend corresponding to a speed of sound of 2900 ± 100 m/s. We provide the first experimental evidence for the existence of two branches also in the dispersion curves of chromatin. The high-energy mode displays an acoustic-like behavior with a sound velocity similar to unstained cells, but in this case the branch likely originates from the superposition of intramolecular DNA optic modes. A low-energy optic-like branch, distinctive of the chromatin moiety, is found at about 2.5 meV.



SECTION: Biophysical Chemistry and Biomolecules

Biomolecules are shown in most textbooks and publications in a static conformation and often naked, without hydration shell and bulk solvent, whereas time-dependent fluctuations are rarely mentioned, even if they are known to span several orders of magnitude, from fractions of a second down to less than 1 ps. Experimental evidence has shown that solvent-coupled protein internal dynamics in the terahertz frequency range is pivotal to biological functionality.[1,2] In particular, the biomolecule close-packed structures entail a widespread internal collective dynamics to enable effective configuration changes toward the active conformation. These motions give rise, among other things, to coherent density fluctuations in the picosecond time-window, which recall the role of phonons in crystals. In biological matter, thermal collective fluctuations are supposed to assist a number of key cellular processes like DNA base-pair opening during replication, transcription, and denaturation,[3,4] in a similar way as phonons often assist structural phase transitions in crystalline systems.

Of course, the dynamical features of biomolecules strongly depend on the environment in which they are embedded. Recent inelastic neutron scattering (INS)[5−8] and molecular dynamics (MD) simulations[9] have been exploited to reveal the coherent terahertz dynamics of biological water. The picture emerging from these studies is twofold. First, there exist collective density fluctuations of biological water similar to those observed in bulk water,[10−13] showing the so-called *fast sound* phenomenon, which consists in a propagation velocity in the terahertz domain in excess of 3000 m/s, i.e., much larger than that of 1500 m/s observed in the megahertz range in bulk water. Second, there is evidence for a lower-frequency mode having an *optic or transverse-like* character as in bulk water,[10−13] which is related to local correlations possibly due to the hydrogen bond network. On the other hand, collective excitations of proteins and DNA in dry and weakly hydrated forms exhibit analogous features as their solvent, as shown by INS[8] and inelastic X-ray scattering (IXS)[14−19] measurements and MD simulations.[20] This similarity between biomolecules and water has been speculated to have a key biological role.[8,19] However, to shed more light into this subject, terahertz coherent fluctuations of biomolecules should be probed in the natural environment where they perform their activity. In a cell, which is the basic unit of life, a high macromolecular concentration is present, and motions in a wide time window are relevant for the system to function. At present, little detailed information on atomic-scale thermal motions in the case where all biological components are embedded in their cellular environment is available.[21−23] As to the collective motions,

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recent Brillouin neutron scattering investigations on *Escherichia coli* bacteria revealed the similarity between the dynamical behavior of the micro-organism as a whole and bulk water.[24,25] One might expect that an important step forward for modern biophysics would be to characterize the dynamics of specific cellular components, such as DNA, in conditions as close as possible to their actual physiological state.

In the present paper, we present an IXS experiment, devoted to probing the terahertz collective dynamics of human cells, U937 lymphocytes, and their chromatin. To enhance the signal coming from chromatin, cells have been stained by uranyl-acetate salt, which is expected to strongly bind to the DNA phosphate groups.[26,27] Here we make the approximation that the collective dynamics of DNA is only marginally affected by the staining with uranyl group because, as on the present sample, we mainly probe optic-like modes (see the Supporting Information). Staining with heavy ions is a standard tool in electron microscopy experiments focused on the cellular DNA and its effects are clearly visible in X-ray diffraction (XRD) data (for details about the staining procedure, see the Supporting Information). In Figure 1, the XRD intensity of the unstained

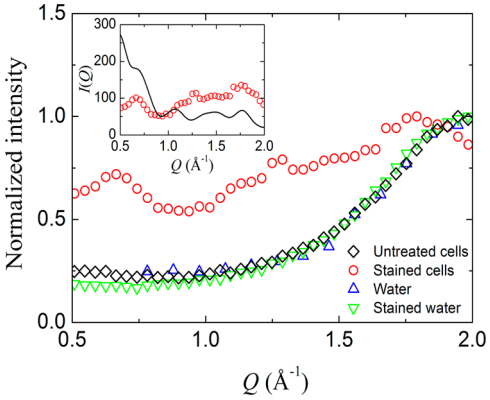

Figure 1. Static structure factor of the measured samples. For the sake of comparison, the data have been rescaled to their main peaks, namely at 2 Å^{-1} for unstained cells, bulk water, and uranyl-acetate solution, and at 1.8 Å^{-1} for stained cells. Inset: data of the stained cells compared with the static structure factor of B-DNA calculated with the Debye formula (isotropic approximation).

cells is almost indistinguishable from that of bulk water, which is the main cellular component, and indeed is dominated by the well-known broad peak of H_2O at about 2 Å^{-1}. Also the diffraction pattern of the uranyl-acetate in water solution exhibits the same shape as bulk water, thus demonstrating that the signal from unbound uranyl ions is rather featureless. On the other hand, the stained cells pattern is strongly different and shows some distinctive features below the main peak at about 1.8 Å^{-1}, namely a small peak at about 0.7 Å^{-1} and a broad shoulder centered at about 1.2 Å^{-1} that almost completely overwhelms another small bump at 1.3 Å^{-1}. In fact, as shown in the inset, these features are qualitatively well reproduced by the static structure factor of the double helix of B-DNA, calculated from the PDB 1BNA coordinates[28] by means of the Debye formula, i.e., in the isotropic approximation (for details, see the Supporting Information).

If we discriminate the contributions from different structural elements of the double helix, we can see that the interference pattern in the Q-range 0.5−1.7 Å^{-1} is dominated by the sugar and phosphate backbone, while the major peak around $Q \approx$ 1.7−2.1 Å^{-1} arises mostly from base-pair stacking. In the calculation we only had to slightly expand the double helix pitch by 8% to correctly reproduce the position of the latter peak, such an effect being probably related to the presence of the large uranyl ions perturbing the usual B-DNA conformation. The small peak at about 1.3 Å^{-1} is likely due to the contribution of other stained cellular components, such as phospholipid membranes whose main diffraction peak at 1.4 Å^{-1} is related to the characteristic distance between hydrocarbon tail groups.[29] Also in this case, a small increase of this characteristic distance due to the interaction with uranyl ions may explain the shift of the peak to lower values. Altogether, the diffraction pattern of treated cells suggests that the staining procedure is quite effective to single out the chromatin signal with respect to the other cellular components.

The IXS spectra of unstained and stained cells are shown in Figure 2 for two different Q values. In the case of stained cells,

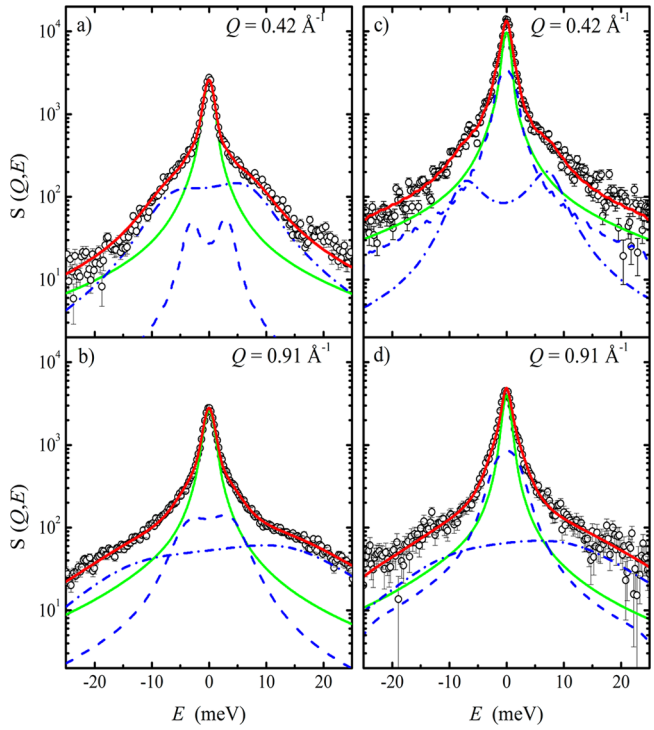

Figure 2. IXS data (black open circles) at two typical Q values, Q = 0.42 (first row) and 0.91 Å^{-1} (second row). Panels a and b report spectra of the unstained cell, while the chromatin data are shown in panels c and d. The red line represents the best fit to the data (see text). Blue lines are the inelastic fit components. The green line is the experimental resolution function.

i.e., panels c and d, even if the signal is dominated by the DNA contribution, we also subtracted the properly rescaled unstained cells intensity to remove the residual signal from components other than chromatin (for details about the subtraction procedure, see the Supporting Information). The measured IXS scattering intensity is directly related to the dynamic structure factor $S(Q,E)$, a function that contains information on the collective dynamics, through the relationship

$$I(Q, E) = R(E) \otimes S(Q, E)\beta E[n(E) + 1] \quad (1)$$

where $R(E)$ is the instrument energy resolution function, $n(E)$ is the Bose factor, and β is the inverse temperature. To exploit the wealth of information embedded in the experimental data,

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the $S(Q,E)$ has been interpreted by means of an empiric model widely used to characterize terahertz collective density fluctuations in bulk water[10−12] and in biological systems, such as dry proteins,[8] hydrated DNA[6,7] and living bacterial cells:[24,25]

$$S(Q,E) = S_{el}(Q,E)a_{el}(Q) + \sum_{i=1}^{n} \frac{a_i}{\pi} \frac{\Gamma_i(Q)E_i^2(Q)}{(E^2 - E_i^2(Q))^2 + (\Gamma_i(Q)E)^2} \quad (2)$$

In this equation, $S_{el}(Q,E)$ accounts for the intensity of the elastic peak and its possible broadening with respect to the experimental resolution due to quasi-elastic contributions. This term has been described as a Lorentzian and a δ-function for unstained cells and chromatin, respectively. The inelastic contributions are assumed to be well described by $N = 2$ damped harmonic oscillators (DHO), having excitation energies $E_i(Q)$ and damping factors $\Gamma_i(Q)$. The coefficients $a_{el}(Q)$, and $a_i(Q)$ account for the amplitudes of the various components. Thanks to the good quality of the measured spectra, we have been able to determine reliable values for the eight free parameters needed to describe the signal of both unstained cells and chromatin. In Figure 2 we show the excellent agreement in the whole energy range between the fitting curves and the experimental data. A first inspection of the spectra suggests that the low-energy component of the inelastic signal from chromatin is significantly red-shifted with respect to that of unstained cells.

For a more quantitative analysis, in Figure 3 we report the corresponding dispersion curves, which are obtained by plotting the excitation energies as a function of Q. In the case of the unstained cells, the high-frequency mode shows a low-Q linear trend that we interpret as an acoustic-like longitudinal excitation, i.e., a sound mode. This dispersion curve is quantitatively very similar to that of bulk[10−13] and biological[5−7,24] water, consistent with the fact that the solvent contribution dominates the inelastic spectra of unstained cells.

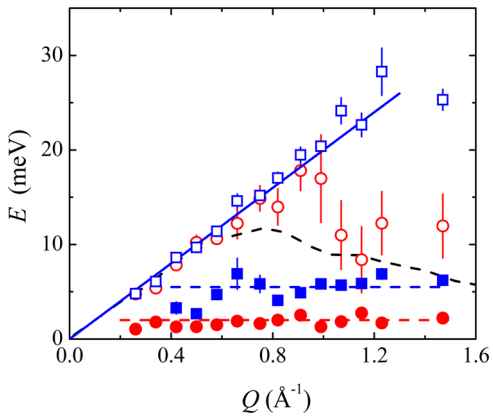

Figure 3. Dispersion curves for the high-frequency (open symbols) and low-frequency (closed symbols) modes of chromatin (circles) and unstained cells (squares). Blue and red lines are fits to unstained cells and chromatin branches, respectively. The black dashed line is the dispersion curve from the normal mode calculations done by Merzel et al. on a fully atomistic model of hydrated DNA.[33]

In more detail, the propagation speed of this excitation is easily obtained by a linear fit on the low-Q data points and turns out to be 2900 ± 100 m/s, which is rather close to the value of 3200 ± 100 m/s found for bulk water.[12] Such high-frequency propagation speed measured by IXS is much higher than the ordinary sound velocity we measured on the same sample by an in-house ultrasound apparatus, which turned out to be 1480 m/s. The discrepancy of about a factor of 2 between the terahertz and the megahertz range velocities recalls the scenario occurring in bulk water, where these propagating excitations are usually distinguished as "fast" and "normal" sound.[10−13] As in the case of pure water,[10−13] we suggest that the high propagation speed of the density fluctuations originates from the fact that, in the picosecond time-window, biological water has a more solid-like dynamical behavior, compared to the normal liquid behavior on the microsecond time-scale. The overall similarity between bulk and biological water indicates that the high-frequency collective dynamics of the latter is only slightly perturbed by the macromolecular crowding of the cellular environment. This small perturbation is visible only in the quadratic Q-dependence of unstained cells damping factors that, at variance with the usual linear trend of the bulk solvent,[10,11] is a signature of collective dynamics of liquids in viscous environment (see Supporting Information).

Such a result is not trivial, as macromolecular crowding is expected to deeply affect the tetrahedral coordination geometry of the extended hydrogen-bond network of water.[30] This behavior of biological water is also quite akin to that of biological molecules, such as dry and hydrated maltose binding protein,[8,31] or DNA at low-hydration conditions. For the latter, previous IXS measurements reported a speed of 2850 m/s in DNA oriented fibers[15] and velocities between 2750 and 3150 m/s for liquid crystalline DNA, depending on the counterion species.[14]

The low-energy mode in unstained cells shows a Q-independent behavior which is localized at an almost constant value of 5 meV. Because the spectra of the unstained cells are dominated by the signal of intracellular water, also this second excitation can be attributed to biological water. Indeed, such an optic-like mode is observed at exactly the same energy in pure water,[10−13] where it was shown to originate from collective modes sustained by intermolecular bending vibrations, perpendicular to the line formed by the hydrogen bond O−H···O.[32]

The dispersion curves of chromatin instead arise mainly from the signal of uranyl ions bound to the phosphate groups of cellular DNA. Quite consistently with this assumption, Figure 3 shows that the trend of the high-frequency mode in chromatin is in good agreement with the IXS results found by Krisch et al. on DNA fibers[15] and with the normal mode calculations done by Merzel et al. on a fully atomistic model of hydrated DNA.[33] This numerical work provides a reasonable interpretation for this branch, which would originate from the projection of the spectral intensity of many optic modes of the helix with polarization in the direction of the DNA fiber axis.[33] The strong Q^2 increase of the damping factor of this chromatin branch (see Supporting Information) is also consistent with the mainly localized nature of the superimposed optic-like modes of which it is composed.[33] As a consequence, the experimental propagation speed of about 2800 m/s, which is very similar to that of unstained cells, may be interpreted as an apparent sound velocity. Interestingly, the chromatin high-energy branch shows a maximum value at about 0.9 Å^{-1}, which is quite similar to the approximately sinusoidal behavior found by Merzel et al. and ascribed to the periodicity of the DNA base-pair rise.[33]

The dispersion curves of chromatin also show an optic-like mode located at a lower energy than unstained cells, namely,

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around 2.5 meV. Such a mode is reminiscent of a low-energy excitation already found by MD simulations, X-ray scattering, and Raman studies on DNA oriented fibers and crystals.[33−36] This mode is strongly affected by the hydration degree of the system and disappears in solution.[35,36] As a consequence, it has been ascribed to intermolecular collective modes involving the motions of the outer part of neighboring helices, i.e., phosphate groups and their associated counterions. On the other hand, normal mode calculations found out a bunch of optic-like excitations centered at about 3 meV, mainly assigned to out-of-plane rocking of nucleotides,[33] that might as well be related to the low-energy branch we revealed in chromatin, although this assignment is inconsistent with the interhelical origin mentioned above. The comparison of the present results with the IXS data and normal mode calculations provides strong evidence that chromatin and simple model systems such as DNA fibers share a quite similar landscape for picosecond collective excitations.

In summary, we report about first measurements of terahertz collective motions in U937 lymphocytes and their chromatin. We have demonstrated that a selective staining with uranyl-acetate salts can be effectively employed to enhance the signal from chromatin.

The collective dynamics of both unstained cells and enhanced chromatin present a double-branched dispersion curve.

In the case of the unstained cells, the dynamics is mainly dominated by biological water, with the dispersion curves in quantitative agreement with those of water. The acoustic branch, with a high-frequency speed of 2900 m/s, shows the existence of the same fast-sound effect; the optic-like mode, originating from transverse hydrogen-bond bending vibrations, lies around 5 meV. We may speculate that the similar terahertz dynamics of biological water and biomolecules can foster low-frequency vibrational coupling between biomolecules and solvent, thus playing a major role in biological activity.[19]

Also, in chromatin, the trend of the high-energy mode displays an acoustic-like behavior, but the comparison with normal mode calculations suggests that in this case the branch comes from the complex superposition of intramolecular DNA optic modes. At variance with the case of unstained cells, the optic-like branch in chromatin is located at the lower energy of 2.5 meV, which seems to be a peculiar characteristic of cellular DNA dynamics related to twisting motions of base-pairs.

Globally, our results indicate that the THz collective dynamics of cellular DNA is independent of perturbation from steric effects due to packing within chromatin and interaction with chromatin proteins.

EXPERIMENTAL METHODS

U937cells, derived from a human hystiocytic lymphoma, were acquired from SIGMA-Aldrich (Sigma-Aldrich, St Louis, MO, USA, L9393). Details of the cells growth, preparation and coloration with uranyl-acetate are reported in the Supporting Information.

XRD measurements were performed using a standard sealed tube X-ray diffractometer operated using the Ag K_α radiation, with the K_α radiation removed by means of a graphite crystal monochromator. The X-ray tube was operated at 50 kV and the half wavelength radiation was removed by a proper pulse height analysis of the detector output.

IXS measurements were performed at the inelastic X-ray scattering beamline ID16 of the European Synchrotron Radiation Facility (Grenoble, France). The (11 11 11) reflection of the silicon crystal monochromator was exploited to select an incident photon energy of 21.747 keV with a resolution of 1.5 meV. Spectra were acquired in an energy window from −40 to 40 meV and a momentum transfer (Q) range from 0.26 to 1.43 A^{-1}. Measurements were performed at 310 K, i.e. at the physiological temperature of the cells.

A detailed description of the experimental setups and data reduction is reported in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

Sample preparation and characterization, sample container, X-ray diffraction measurements and data analysis, computation of the DNA diffraction pattern, inelastic X-ray scattering measurements and data analysis, and damping factors analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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