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# Collective Dynamics of Intracellular Water in Living Cells

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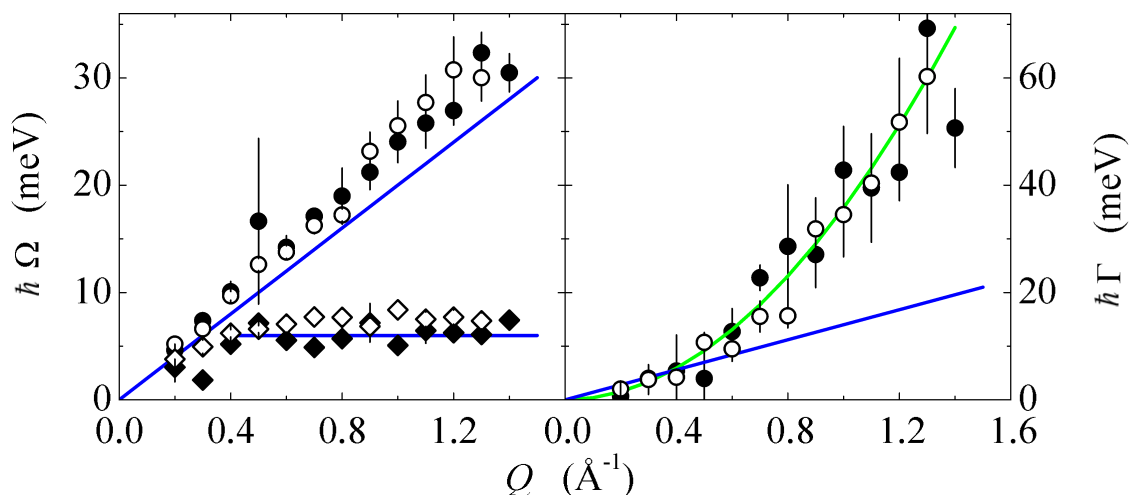
**Abstract.** Water dynamics plays a fundamental role for the fulfillment of biological functions in living organisms. Decades of hydrated protein powder studies have revealed the peculiar dynamical properties of hydration water with respect to pure water, due to close coupling interactions with the macromolecule. In such a framework, we have studied coherent collective dynamics in protein and DNA hydration water. State-of-the-art neutron instrumentation has allowed us to observe the propagation of coherent density fluctuations within the hydration shell of the biomolecules. The corresponding dispersion curves resulted to be only slightly affected by the coupling with the macromolecules. Nevertheless, the effects of the interaction appeared as a marked increase of the mode damping factors, which suggested a destructuring of the water hydrogen-bond network. Such results were interpreted as the signature of a “glassy” dynamical character of macromolecule hydration water, in agreement with indications from measurements of the density of vibrational states. Extending the investigations to living organisms at physiological conditions, we present here an *in-vivo* study of collective dynamics of intracellular water in *Escherichia coli* cells. The cells and water were fully deuterated to minimise the incoherent neutron scattering background. The water dynamics observed in the living cells is discussed in terms of the dynamics of pure bulk water and that of hydration water measured in powder samples.

## 1. Introduction

Water dynamics is well known to play a crucial role for the fulfillment of biological functions in living organisms. Indeed biological macromolecules, like proteins or nucleic acids, can accomplish their functional tasks only if allowed to explore the vast landscape of their conformational substates through thermal anharmonic atomic movements. Such macromolecular dynamics, mainly occurring on the picosecond time scale, are practically frozen out in dry samples and become active only in the presence of a sufficient quantity of hydration water, which provides the macromolecule with the necessary structural flexibility. Within this framework, the achievement of a better understanding of the dynamical behaviour of hydration water itself is obviously a challenge of major interest [1].

A vast literature exists on single-particle thermal motions of hydration water, which can involve diffusion phenomena of translational or rotational nature. The large majority of investigations by different methods have shown that water diffusion in a hydration shell extending over one or two molecular layers is significantly hindered by interactions with a biomolecular surface, resulting for example in diffusion coefficients a factor 2 to 10 smaller than in bulk water (see for instance Ref. [2]). Measurements of single-particle water motions in *Escherichia coli* (*E. coli*) [3, 4] and red blood cells [5] have revealed that a major fraction of approximately 80-90% of water in these cells is characterized by a translational diffusion coefficient similar to bulk water. A minor fraction of approximately 10% of cellular water, which exhibits reduced dynamics, was attributed to dynamically bound water on the surface of the macromolecules. On the other hand, collective density fluctuations in the hydration shell of biomolecules have started to draw some attention only recently, despite the propagation of such excitations might be involved in a number of biological actions requiring transmission of information, like for instance allosteric reactions. The study of collective modes on the picosecond time scale has been significantly boosted by the advent of Brillouin spectroscopies at THz frequencies, which can nowadays be achieved by inelastic neutron and X-ray scattering (INS and IXS respectively).

The state-of-art neutron Brillouin spectrometer BRISP [6] has allowed our group to undertake a systematic investigation of collective density fluctuations in various biological samples, with particular focus onto the dynamical behaviour of hydration water. The determination of THz dispersion curves for the hydration shell of a biomolecule was first achieved for the Ribonuclease A (RNase) protein [7], at hydration levels between 0.7 and 1.0 g of water per g of protein. In this system, two collective modes of different characteristic energies  $\hbar\omega$  (or frequencies  $\omega$ ) were observed in the wavevector range  $Q = 0.2 - 1.4 \text{ \AA}^{-1}$ . As shown in the left panel of Fig. 1, the so-called low-frequency mode displays an optic-like character with a rather constant energy of about 6-7 meV. The so-called high-frequency mode is instead considered of acoustic-like nature and is characterised by linearly increasing energies with



**Figure 1.** *Left:* Experimental dispersion curves of THz collective modes in RNase (full symbols) and DNA (empty symbols) hydration water. Circles refer to the high-frequency mode, lozenges to the low-frequency mode. The dispersion curves of bulk water (full lines) are reported for comparison. *Right:* Damping factors of the high-frequency mode for RNase (full circles) and DNA (empty circles) hydration water. The green solid line is a quadratic fit of the damping factors  $Q$ -dependence. The blue straight line represents the trend of the high-frequency damping factor in bulk water.

increasing wavevector: the low- $Q$  slope of this mode provides a propagation speed of about 3400 m/s. Both excitations are strongly damped. The high-frequency mode, in particular, shows a  $Q^2$  increase of the damping factor (right panel of Fig. 1), which crosses the corresponding dispersion curve at about  $0.6 \text{ \AA}^{-1}$  (not shown). For higher  $Q$ -values, the density fluctuation becomes overdamped and consequently its propagation becomes more and more hindered and short-ranged. Very recently we have extended such studies to samples of hydrated DNA, at hydration levels between 1 and 15 g of water per g of DNA [8, 9]. Despite the different structural conformation and hydrophilic sites distribution of the DNA helix with respect to the globular protein RNase, the collective dynamics of hydration water behaves very similarly in the two systems. As shown in Fig. 1, the same two-mode dispersion curve, with similar characteristic energies,  $Q$ -dependences, propagation speeds and damping factors, is observed in the hydration water of both macromolecules.

Such a double-branched structure of the THz dispersion curve closely resembles what observed in pure water by both INS and IXS [10, 11, 12] (see Fig. 1). In the bulk liquid, the low-frequency mode is located at the same almost-constant energy of  $\sim 6$  meV, while the high-frequency one propagates at the slightly lower speed of  $3040 \pm 80$  m/s. Nonetheless, the major difference between bulk and hydration water seems to reside in the damping factors amplitude and  $Q$ -dependence. Indeed, the damping factors of hydration water are larger than in the bulk and increase with a parabolic, rather than linear [10, 11], wavevector dependence. Such larger damping factors correspond to shorter propagation lifetimes and mean free paths of the collective modes, which is ascribed to a destructuring effect on the local tetrahedral order of water hydrogen bonds, induced by the interaction with the biomolecule surface [7]. Such a perturbation of the hydrogen-bond network confers on hydration water a “glassy” dynamical character, as also indicated by previous determinations of the density of vibrational states [13].

As a whole, the emerging picture of hydration water dynamics suggests that the interaction with the biomolecule produces, on one hand, a remarkable slowing down of diffusional single-particle processes and, on the other hand, a sizeable reduction of the propagation lengths of coherent collective modes, despite the speed and frequencies are only slightly affected.

In this framework, the characterisation of intracellular water collective dynamics in *E.coli* is complementary to the single-particle dynamics results obtained from quasi-elastic incoherent neutron scattering on both *E.coli* and red blood cells, which have shown that single-particle water motions beyond the macromolecule hydration shells were similar to bulk water [3, 5]. Consequently it appears interesting to consider whether a similar effect takes place for the collective dynamics as well. In this short paper, we present the preliminary results of a neutron Brillouin experiment performed on a culture of living *E.coli* bacteria, with the aim of observing whether the collective dynamics of intracellular water appears closer to that of the pure liquid or of the hydration layer of protein and DNA powders.

## 2. Experimental Methods

Due to the large incoherent scattering cross-section of hydrogen, with respect to the mainly coherent cross-section of deuterium, a neutron spectroscopy investigation on the coherent dynamics of cellular water requires the availability of a fully deuterated sample of living cells.

To this end, a fermenter culture of fully deuterated *E.coli* bacteria was grown at the Deuteration Laboratory of the Institut Laue-Langevin (ILL). From the fermenter culture, about 7 g of living *E.coli* were harvested by centrifugation, sealed in a slab-shaped aluminium mount ( $30\text{mm} \times 50\text{mm} \times 4\text{mm}$ ) and placed in the neutron beam of the BRISP spectrometer at ILL. The centrifugation step was carefully controlled to get rid of the maximum amount of external water, yet without removing any intracellular water, which would destroy the cell envelope of *E.coli* and kill the cell. After the experiment, the dry weight of the sample was measured to be 20% of the total. Of the 80% water weight, 8% corresponded to extracellular and 72% to intracellular

water [14]. Storage of the deuterated bacterial cell paste at room temperature for about 48 hours did not lead to any significant cell death. To ensure that the full set of measurements were performed on actually living cells, the sample in the neutron beam was replaced with a fresh one taken from the cell culture every 24 hours. The experiment lasted 7 days, 4 of which were devoted to the *E.coli* cells and the remaining 3 were employed for background, empty sample holder and vanadium standard measurements. All measurements were performed at room temperature. The BRISP spectrometer was used with an incident wavelength of 0.9885 Å and a sample-to-detector distance of 4.5 m.

The true scattering intensity  $I(Q, \omega)$  of the *E.coli* cells was obtained from the raw data by following standard reduction procedures. In particular, the data were corrected for environmental and sample holder contributions, energy dependence of the detector efficiency, sample self-absorption and multiple scattering. The resulting spectra at selected  $Q$ -values are plotted in Fig. 2. Because of the fully deuterated nature of the samples, to a first approximation the residual incoherent contributions can be neglected. In addition, considering the very high water content in the *E.coli* cell, in the present very preliminary analysis the spectra in Fig. 2 will be ascribed to the dynamical behaviour of intracellular water only, while the contribution of the “dry” *E.coli* components will be neglected. More detailed and specific analyses are under development and will be published elsewhere.

### 3. Data Analysis and Discussion

For a quantitative analysis of the data, the spectra  $I(Q, \omega)$  can be fitted to a model function based on a classical dynamic structure factor  $S_{cl}(Q, \omega)$ , properly multiplied by the Bose-Einstein occupation number  $n(\omega, T)$  and convoluted with the energy resolution function  $R(\omega)$ :

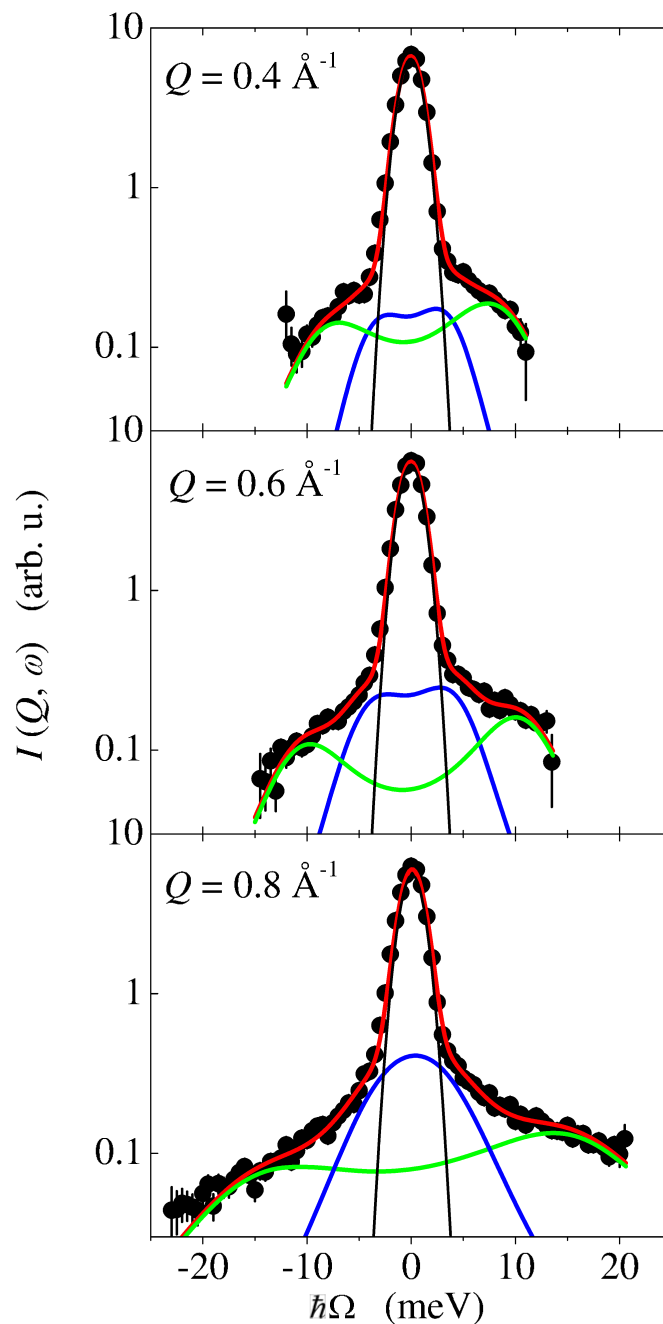
$$I(Q, \omega) = [I_0(Q)R(\omega)] \otimes \left\{ \frac{k_f}{k_i} \frac{\hbar\omega}{k_B T} [n(\omega, T) + 1] S_{cl}(Q, \omega) \right\}, \quad (1)$$

where  $k_f$  and  $k_i$  are the final and incoming neutron wavevector respectively,  $T$  is the absolute temperature,  $k_B$  is the Boltzmann constant and  $I_0(Q)$  is an overall scaling factor. At the employed instrumental configuration, the BRISP resolution function  $R(\omega)$  had a Gaussian shape with a full width at half maximum of 2.7 meV, as determined by the vanadium measurement. The choice of the most suited form for the dynamic structure factor  $S_{cl}(Q, \omega)$  depends of course on physical considerations about the system under investigation.

From a first look at  $I(Q, \omega)$  in Fig. 2, it emerges that clear inelastic features appear in the spectra well outside the experimental resolution function. Based on the approximations discussed in the previous section, we interpret such features as coherent collective modes propagating through *E.coli* intracellular water. As recalled in the Introduction, all previous determinations of collective density fluctuations in either pure [10, 12] or interacting water [7, 15] report the existence of two inelastic modes. In addition, to a closer inspection of the present spectra, the two modes are already visible in the raw data at intermediate  $Q$ -values (e.g. 0.6 Å<sup>-1</sup>). On such grounds, it appears reasonable to chose a dynamic structure  $S_{cl}(Q, \omega)$  containing two damped harmonic oscillators (DHOs):

$$\begin{aligned} S_{cl}(Q, \omega) = & A_{el}(Q)\delta(Q, \omega) + \\ & + \frac{A_L(Q)}{\pi} \frac{\Omega_L^2(Q)\Gamma_L(Q)}{[\omega^2 - \Omega_L^2(Q)]^2 + \omega^2\Gamma_L^2(Q)} + \\ & + \frac{A_H(Q)}{\pi} \frac{\Omega_H^2(Q)\Gamma_H(Q)}{[\omega^2 - \Omega_H^2(Q)]^2 + \omega^2\Gamma_H^2(Q)}, \end{aligned} \quad (2)$$

where the subscripts refer to the low ( $L$ ) and high ( $H$ ) frequency mode, while  $\Omega(Q)$ ,  $\Gamma(Q)$  and  $A(Q)$  represent respectively the proper frequencies, damping factors and intensity factors of

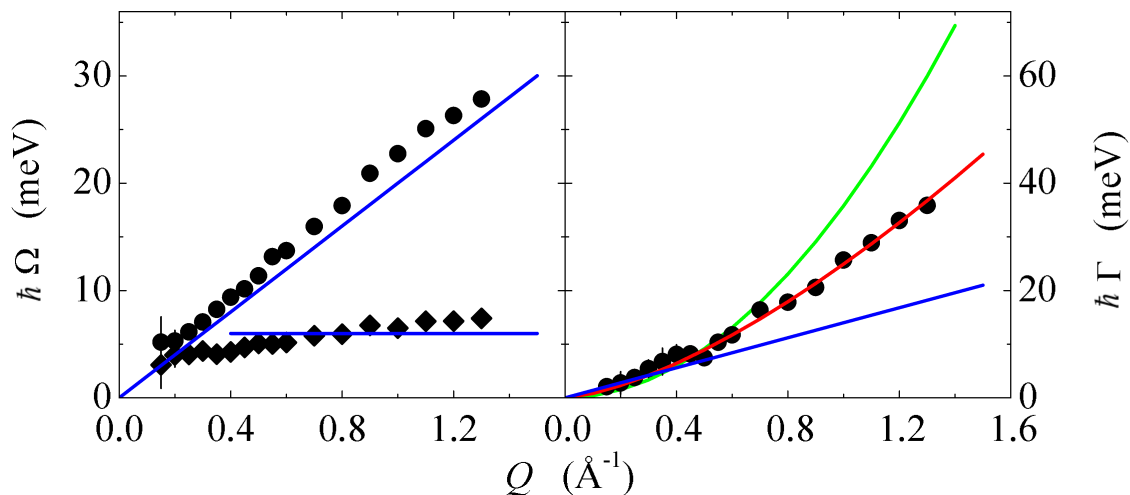


**Figure 2.** Coherent inelastic spectra of *E. coli* intracellular water at selected  $Q$ -values. The black line shows the experimental resolution function. Inelastic shoulders are well-visible outside the resolution function. The red line shows a fit of the data with two DHOs (see text). The blue and green lines represent respectively the resulting low- and high-frequency DHO.

each DHO. The first term containing a Dirac  $\delta$ -function accounts for the quasielastic peak of the sample. Due to the relatively large energy resolution of the present experiment, a more specific definition of the quasielastic lineshape is not needed. The fits of the experimental  $I(Q, \omega)$  with the model of Eqs. 1 and 2 were performed independently at each  $Q$ -value. The best fitting functions are shown in Fig. 2. It is readily seen that the fits are in excellent agreement with the measured spectra, thus supporting the existence of two well defined modes. The resulting oscillation frequencies  $\hbar\Omega$  and damping factors  $\hbar\Gamma$  are plotted in Fig. 3 as functions of  $Q$ .

The dispersion curves of intracellular water closely recall those of bulk water and display an even stronger resemblance to those of hydration water of DNA and RNase (cfr. Fig. 1). The low-frequency mode, with an almost constant characteristic energy, is located between 4 and 7 meV, slightly increasing with  $Q$ . The high-frequency mode has a fairly linear  $Q$ -dependence, with a steady slope corresponding to a propagation speed of  $3460 \pm 30$  m/s. Similarly to what observed for protein and DNA hydration water, such results indicate that interactions with the biomolecular environment inside *E.coli* cells produce only minor changes in the THz dispersion curves of water.

A more interesting piece of information is brought about by the behaviour of the damping factors, in particular those concerning the high-frequency mode. The right panel of Fig. 3 shows that, although the values of  $\hbar\Gamma_H$  increase steadily faster than the linear behaviour of bulk water, they remain well below the quadratic trend observed for RNase and DNA hydration water. Indeed, a fit of  $\hbar\Gamma_H$  to a power law of the form  $aQ^x$  yields for the exponent the result  $x = 1.5 \pm 0.1$ , to be compared with  $x = 2$  for hydration water and  $x = 1$  for bulk water. As recalled in the Introduction, the quadratic damping factors of the solvent layer surrounding DNA and RNase are interpreted in terms of shorter propagation lengths due to a “glassy” dynamic character of hydration water. In view of such a picture, the observed trend of  $\hbar\Gamma_H$  in *E.coli* suggests that intracellular water might be regarded as a mixture of both bulk and hydration water. Water molecules in close contact with proteins, nucleic acids, or other macromolecular



**Figure 3.** *Left:* Experimental dispersion curves of THz collective modes of *E.coli* intracellular water. Circles refer to the high-frequency mode, lozenges to the low-frequency mode. The dispersion curves of bulk water (full lines) are reported for comparison. *Right:* Damping factors of the high-frequency mode for *E.coli* intracellular water (circles). The red solid line is a fit to the high-frequency damping factor with a  $Q^{1.5}$  power law. The green and blue solid lines report the behaviour of hydration and bulk water respectively.

constituents of the *E.coli* interior would display the dynamical character of hydration water. The remainder of water would instead behave like the free normal liquid, in qualitative agreement with what found for the single-particle dynamics counterpart [3].

This hypothesis might be pushed further by considering the damping factors of *E.coli* water like a weighted average of the damping factors of bulk and hydration water. Such an average can be calculated by using the present damping factors data to work out an experimental estimate of the fraction of free water in *E.coli* cells. At the various  $Q$  values, it turns out that the fraction of normal water is about 0.6 with respect to the total amount of intracellular water. If one considers that the *E.coli* cell is composed by intracellular water for approximately 72% in weight [14], the above-estimated fraction of free liquid suggests that the remaining interacting water hydrates the cell inner components to a hydration level of about 0.9, which would approximately correspond to three layers of coordinated water around a protein or biomolecule in the cell. The fraction of intracellular water whose collective dynamics is affected by macromolecular interactions is higher than found previously for single-particle motions [3, 4]. Considering the approximations involved in the calculations, however, further experiments will be necessary before the significance of the difference is established.

#### 4. Conclusion

The present experimental study reports about the observation by neutron Brillouin spectroscopy of THz collective modes propagating in *E.coli* intracellular water. Preliminary results indicate that, although the dispersion curves resemble those of protein and DNA hydration water, the corresponding damping factors suggest intracellular water to be composed by a considerable amount of free non-interacting water. This finding complements and corroborates the results recently obtained for the single-particle dynamics of *E.coli* water [3].

A more detailed and precise analysis should take into account the role of density fluctuations propagating through the cell itself. This necessarily involves a careful separation of the experimental  $I(Q, \omega)$  into partial dynamic structure factors, separately accounting for water-water, cell-cell and water-cell correlations. Although a reliable such procedure requires considerable efforts in terms of data reduction and analysis, work toward this direction is currently in progress.

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