Atomic-scale dynamics inside living cells explored by neutron scattering

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Single-particle neutron spectroscopy has contributed important experimental data on molecular dynamics in biological systems. The technique provides information on atomic and molecular motions in macromolecules on the picosecond to the nanosecond time scale, which are essential to biological function. Here, we report on recent neutron measurements performed directly in living cells by using isotope labelling to explore the dynamics of specific cellular components. The paper proposes an integrated view of results on atomic-scale cell water dynamics, internal and global macromolecular motions and solvent isotope effect on macromolecular dynamics. The work established the specific usefulness of the neutron scattering technique to get insight into biologically relevant dynamical features, in particular through comparative measurements. The method developed can now be applied to look for dynamical signatures related to cell characteristics in many different cell types and organelles.

Keywords: neutron scattering; living cell; molecular dynamics; picosecond–nanosecond time scale; cell components

1. THE CELL, A DYNAMIC AQUEOUS ENVIRONMENT

The cell is the fundamental unit of life. It is a highly evolved system with a level of complexity that drives many open questions. Obtaining *in vivo* information at the molecular scale is challenging. One of the essential properties of the cell is its dynamic nature. At every length scale of matter, the cell is in constant motion, and the different length and time scales are closely connected. For example, cell motility and cell cycle are possible through molecular interactions in the dynamic cytoskeleton network. Actin polymerization is used to produce protrusive and contractile arrays that cooperate to drive cell motility (Small & Resch 2005). In interphase cells, actin arrays are also involved in pre-mitotic nuclear movement (Kennard & Cleary 1997) and nuclear movement following division (Kost & Chua 2002). More generally, biological macromolecules are animated by a large variety of motions on a wide range of time scales, going from the femtosecond to the second, and arising on various length scales. Proteins experience different conformations separated from each other by small structural changes on fast time scales. Atomic and molecular motions on the picosecond to the nanosecond (ps–ns) time scale lubricate larger conformational changes on slower, millisecond, time scales, which are necessary for important biological processes, including enzyme catalysis and intermolecular recognition

(Jimenez *et al.* 2004; Tousignant & Pelletier 2004). Depending on the conformation, proteins will be functional or not.

Water makes up approximately 70 per cent of the cell mass. Water surrounds biological macromolecules, allows them to be stable and active, to interact with each other and to move inside cell cytoplasm and in membranes through lipid headgroup- and protein-hydration interactions. It also constitutes an essential dynamic component of the cell interior, and controversies about its dynamical state have animated the biological community for decades. The perception obtained from indirect experimental observations, which has influenced current thought in significant parts of the biology community, is that the cell interior is a gel or colloidal-like structure in which confined water is interfacial with properties that are significantly different from those of pure water. A 2006 book edited by Pollack *et al.* states in the preface ‘... practically all cell water is interfacial’ (Pollack *et al.* 2006). The claim, however, remains controversial as reviewed by Ball (2008). The apparent diffusion coefficient of water in biological tissues and in the brain has been measured by nuclear magnetic resonance (NMR). The measurements revealed that cell water diffusion, on a micrometric scale, is reduced by a factor of between 2 and 10 compared with pure water. The reduction could be explained partly by tortuosity effects, macromolecular crowding and confinement effects (reviewed by Le Bihan 2007). In contrast, very little is known about cell water dynamics on sub-micrometric and subnanosecond scales. To this day, only a few techniques can be applied, mainly NMR spectroscopy and neutron scattering. These methods

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constitute the key experimental techniques that are capable of providing a detailed view of cell water dynamics.

2. NEUTRON SCATTERING TECHNIQUE, COUPLED WITH ISOTOPIC LABELLING

Experimental molecular dynamics studies in biological systems are attracting growing interest. Single-particle neutron spectroscopy has revealed itself as an extremely rich technique for dynamics measurements on living cells. The method provides a unique combination of atomic length-scale resolution and subnanosecond time-scale resolution. Thermal neutrons provide wavelengths of a few angströms that correspond to interatomic distances, and energies of a few millielectron volts that correspond to thermal fluctuations in biological samples. In these conditions and because of the absence of charge of the neutron, the neutron–matter interaction is non-destructive. Single-particle neutron spectroscopy provides a direct measurement of atomic and molecular motions on the ps–ns time scale.

The scattering signal is dominated by the scattering of hydrogen nuclei, which have an incoherent cross section one order of magnitude larger than the cross section of any other nucleus or isotope, including deuterium. The particularity is very useful to probe the dynamics of biological macromolecules as well as the motions of the surrounding water. The dynamics of a specific part of a complex system can also be enhanced by taking advantage of selective isotopic hydrogen–deuterium (H–D) labelling. Isotope labelling provides a key advantage of the neutron scattering method. A deuteration facility is provided at the Institut Laue-Langevin (ILL) to the users to help them with the preparation of specifically labelled samples, from entire cells to purified components such as proteins or membranes (see <http://www.ill.eu/sites/deuteration/index.htm> for further information).

Single-particle neutron spectroscopy relies mainly on two types of techniques: elastic (EINS) and quasi-elastic (QENS) incoherent neutron scattering. In both experiments, the interference observed is that between an individual nucleus and itself at different positions as it moves in time. The information obtained is that of individual dynamics averaged over all nuclei in the sample, each contributing according to its scattering power (H nuclei will dominate). For this reason and to avoid confusion with incoherence in X-ray or electron diffraction, in the case of neutrons, we shall refer to the process as single-particle spectroscopy.

EINS is measured as a function of the scattering vector, Q . EINS experiments provide information on atomic mean square displacements (MSDs), $\langle u^2 \rangle$, in a sample as a function of temperature, T (Zaccai 2000). The time scale examined depends on the energy resolution of the spectrometer, while the scattering vector, Q , range, in which the scattered intensity is measured, defines the length scale. A structural resilience, expressed as a mean effective force constant (in newtons per metre), $\langle k' \rangle$, can be calculated from the

inverse of the slope of $\langle u^2 \rangle$ versus T (Zaccai 2000; Bicout & Zaccai 2001).

QENS is measured as a function of both energy transfer and scattering vector. QENS provides information on diffusive motions in biological systems (Bée 1988). On the energy scale, the QENS appears as a broad peak centred on zero energy transfer. In the case of simple exponential diffusion processes, the QENS spectra can be fitted mathematically by Lorentzian functions. The dependence of the Lorentzian half-width at half-maximum (HWHM) on Q contains information on the diffusion coefficient and the characteristic time of the motion.

3. ATOMIC-SCALE DYNAMICS INSIDE LIVING *ESCHERICHIA COLI*

3.1. Cell water dynamics

With approximately 70 per cent of the total cell mass, water constitutes the malleable matrix in which macromolecules move and interact. Understanding the dynamic state of water in the cell interior is a major scientific challenge. Recently, water diffusive motions have been explored inside several types of cells, using neutron scattering and isotope labelling (Tehei *et al.* 2007; Jasnin *et al.* 2008a; Stadler *et al.* 2008). The study on *E. coli* shed light on the translational and rotational diffusive motions of water in the bacterial cytoplasm (Jasnin *et al.* 2008a). The experiments were performed on an atomic length scale, and on the picosecond to a few tens of picoseconds time scale. The translational diffusion parameters were extracted from the HWHM of the translational Lorentzian, Γ_T , as a function of the scattering vector squared, Q^2 , at two temperatures (figure 1a). Γ_T was best fitted using a jump-diffusion model, which describes diffusion between sites for the water protons with a mean residence time at each site (Bée 1988). Translational diffusion coefficients were found to be very close to those of pure water at corresponding temperatures, with residence times about twice longer (Teixeira *et al.* 1985; Bellissent-Funel *et al.* 1995). The higher residence times may reflect the longer times spent by the protons in the first hydration shell. The rotational parameters were extracted from the HWHM of the broad Lorentzian, $\Gamma_R + \Gamma_T$, as a function of Q^2 , at two temperatures (figure 1b). The rotational correlation times, $\tau_{\text{cor,R}} = 1/\Gamma_R$, were close to the values extracted for the buffer under the same conditions and of the same order as the values measured for pure water (Sposito 1981; Teixeira *et al.* 1985). We concluded, therefore, that proton exchange between water layers takes place with the diffusion rates of pure-like water. The results established firmly that *E. coli* water diffusion was neither confined nor significantly slowed down compared with pure water. Cell water diffusion beyond the first hydration shell is similar to that of pure water at physiological temperature. The same conclusion has been reached using NMR spectroscopy by the group of Halle (Persson & Halle 2008). They have examined the rotational spin relaxation rate of *E. coli* water over a wide time range from the millisecond to

Figure 1. Translational and rotational diffusive motions of *E. coli* water. (a) HWHM of the translational Lorentzian, Γ_T , as a function of the scattering vector squared, Q^2 , at 281 (squares) and 301 K (triangles). Γ_T was best fitted (solid lines) using a jump-diffusion model, which describes diffusion between sites for the water protons, with a mean residence time at each site (Bée 1988). The translational diffusion coefficients were similar to those of pure water at corresponding temperatures, with residence times about twice longer. The higher residence times may reflect the longer times spent by the protons in the first hydration shell. (b) HWHM of the broad Lorentzian, $\Gamma_R + \Gamma_T$, as a function of Q^2 , at 281 (squares) and 301 K (triangles). The rotational correlation times, $\tau_{\text{cor,R}} = 1/\Gamma_R$, were close to the values extracted for the buffer under the same conditions and of the same order as the values measured for pure water. We concluded, therefore, that proton exchange between water layers takes place with the diffusion rates of pure-like water.

the picosecond time scale. They have found that approximately 85 per cent of *E. coli* water presented rotational relaxation times similar to those of pure water. The studies provided new pieces of data that enlightened cell water dynamics at the molecular scale.

Similar studies were conducted on *Haloarcula marismortui*, an extreme halophilic organism from the Dead Sea, by using neutron scattering (Tehei *et al.* 2007) and NMR (Persson & Halle 2008), respectively. The studies revealed interesting differences between the translational and rotational motions of *H. marismortui* water. The neutron work showed that a significant part of the cell water presented a translational diffusion slowed down by two orders of magnitude compared with pure water (Tehei *et al.* 2007). The NMR work established that the rotational spin relaxation rate of *H. marismortui* water was very close to that found in pure water (Persson & Halle 2008). Both results are not in contradiction and could be partly explained by the high proportion of carboxyl groups along the halophilic structures and the high amount of intracellular K^+ bound within them, which participate in very strong protein–salt ion–water interactions along halophilic protein surfaces (Madern *et al.* 2000). The difference observed between the two types of diffusive motions enhanced the importance of combining different experimental methods to draw a complete view of dynamics in complex systems such as entire cells.

3.2. Internal and global macromolecular motions in *Escherichia coli*

QENS has been used to study internal molecular motions in hydrated protein powders (Doster *et al.* 1989; Andreani *et al.* 1995; Zanotti *et al.* 1997; Fitter 1999; Pérez *et al.* 1999; Dellerue *et al.* 2001; Paciaroni *et al.* 2003; Roh *et al.* 2006) and in the integral membrane protein bacteriorhodopsin in purple membrane stacks (Fitter *et al.* 1996a,b, 1997, 1998, 1999).

Recently, we presented a dynamical mapping of macromolecular dynamics *in vivo*, on the full ps–ns time domain accessible through neutron scattering (Jasnin *et al.* 2008b). The study was performed on *E. coli* cells close to physiological conditions of temperature. The analysis allowed the separation of global and internal dynamical processes, by using a combination of different neutron spectrometers (figure 2). The apparent global macromolecular diffusion in the *E. coli* cytoplasm was extracted from the HWHM of the associated Lorentzian, Γ_{sd} , at two temperatures (figure 2a). We found a translational diffusion coefficient, $D = (1.06 \pm 0.11) \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ at 303 K, which was consistent with the value of $1.2 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ found for haemoglobin in red blood cells at 310 K (Doster & Longeville 2007). Physiological internal motions were found to be a combination of localized reorientational and diffusive motions, occurring from a few picoseconds to approximately 100 ps. The motions observed in a few picoseconds corresponded to restricted jump-diffusion motions, as revealed by the profile of the HWHM of the associated Lorentzian, Γ_f , as a function of Q^2 (figure 2b). They can arise, for example, from the contribution of small groups such as methyl groups, as well as from hydrogen bond breaking and formation in macromolecular structures. Localized reorientational motions emerged on longer, a few tens of picoseconds, time scales (see figure 2c,d and its caption for the details of the analysis). They arise from all protons participating in the rotational motions of small groups (such as protons in methyl groups), in stochastic reorientations of large molecular subunits (such as polypeptide side chains, fatty acid chains or other molecular subunits) as well as in librations of buried groups, relative displacements of globular domains, sugar conformational changes or RNA global bending (McCammon & Harvey 1987).

The comparison with previous *in vitro* work revealed differences between physiological internal motions and

Figure 2. Global and internal macromolecular motions in *E. coli*. The data were collected using three neutron spectrometers at the ILL and ISIS neutron sources. (a) Global motions. HWHM of the Lorentzian associated with the apparent self-diffusion of macromolecules, Γ_{sd} , as a function of Q^2 at 284 (squares) and 303 K (triangles). The Γ_{sd} profile revealed long-range translational motions, as indicated by the increase in the linewidth with Q and the extrapolation to 0 for $Q \rightarrow 0$. We found a translational diffusion coefficient, $D = (1.06 \pm 0.11) \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ at 303 K, which was similar to the value of $1.2 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ found for haemoglobin in red blood cells at 310 K (Doster & Longeville 2007). (b) Localized diffusive motions. HWHM of the Lorentzian associated to 'fast', a few picoseconds, motions, Γ_f , as a function of Q^2 , at 280 (squares) and 300 K (triangles). The Γ_f profile revealed restricted jump-diffusion motions, occurring in approximately 4 ps as given by the value of the plateau at high Q . (c,d) Localized reorientational motions. (c) HWHM of the Lorentzian associated to 'slow', a few tens of picoseconds, motions, Γ_s , as a function of Q^2 , at 280 (squares) and 300 K (triangles). (d) HWHM of the Lorentzian associated to 'very slow', a few hundred picoseconds, motions, Γ_{vs} , as a function of Q^2 , at 284 (squares) and 303 K (triangles).

the motions extracted in hydrated powders and in solution (Zanotti *et al.* 1997; Fitter *et al.* 1998, 1999; Pérez *et al.* 1999; Appavou *et al.* 2006; Tehei *et al.* 2006). On the picosecond time domain, both internal molecular flexibility and diffusion rates are increased in the cell interior compared with the parameters extracted from fully hydrated powders (Zanotti *et al.* 1997; Fitter *et al.* 1998, 1999; Pérez *et al.* 1999). The result showed that the large amount of cell water contributes to picosecond internal macromolecular motions. It suggested that the cell water hydrogen-bond network influences the hydrogen-bond dynamics in macromolecular structures and contributes to physiological structural flexibility on the picosecond time scale. In contrast, picosecond internal flexibility measured in *E. coli* was found reduced when compared with that measured in solution (Pérez *et al.* 1999; Appavou *et al.* 2006; Tehei *et al.* 2006). The result indicated that weak forces owing to the vicinity of macromolecules may attenuate the lubricating effect of cell water. The exploration of *in vivo* dynamics appears all the more crucial because the work revealed that standard sample preparations, such as powders and solutions, do not represent accurately the physiological environment, whose complexity participates in

the functional dynamics necessary for biological activity.

3.3. Solvent isotope effect on macromolecular dynamics

Solvent interactions are essential for macromolecular stability through hydration, hydrogen bonds, van der Waals interactions, ion binding and the hydrophobic effect. Macromolecular folding is the result of a delicate balance between hydration and intramolecular interactions. Heavy water (D_2O) is often used in biophysical studies to replace natural abundance water (H_2O) to prevent signal contamination by water H nuclei or to study exchange or other isotope effects. The differences in properties between H_2O and D_2O molecules, however, are expected to influence macromolecular stabilization and dynamics. The few studies carried out to date in solution have reported that dynamics–stability relationships depend on the protein type. Halophilic proteins were shown to have higher effective resilience in D_2O (Tehei *et al.* 2001), which can be explained by the particularly strong interactions with hydrated salt ions. In contrast, in the case of bovine serum albumin (BSA), heavy water was found to reduce

Figure 3. Solvent isotope effect on macromolecular dynamics in *E. coli*. MSD, $\langle u^2 \rangle$, as a function of temperature, T , from *E. coli* containing H_2O (triangles) and *E. coli* containing D_2O (squares). Macromolecular flexibility, expressed as $\langle u^2 \rangle$ values, is smaller in D_2O . Structural resilience (Zaccai 2000), corresponding to a mean effective force constant, $\langle k' \rangle$, was extracted from the inverse of the slope of $\langle u^2 \rangle$ as a function of T in the two solvent conditions. $\langle k' \rangle$ was found to be smaller in D_2O than in H_2O .

structural resilience (Tehei *et al.* 2001), which suggested a bigger role of entropy in D_2O . Tehei and co-workers have also shown that stability was increased for the two types of proteins in D_2O , revealing that higher stability was not necessarily associated with higher resilience.

We explored the solvent isotope effect on average macromolecular dynamics in the *E. coli* interior (Jasnin *et al.* 2008c). Measurements were performed on living *E. coli* containing H_2O and D_2O , respectively, close to physiological conditions of temperature. Both flexibility, expressed as $\langle u^2 \rangle$ values, and structural resilience, expressed as $\langle k' \rangle$ values, were found to be smaller in D_2O than in H_2O (figure 3). A difference was expected because the driving forces behind macromolecular stabilization and dynamics are different in H_2O and D_2O . In D_2O , the hydrophobic effect is known to be stronger than in H_2O : it favours the burial of non-polar surface groups as well as their van der Waals packing in the macromolecule core. This may lead to the observed smaller $\langle u^2 \rangle$ values. The smaller resilience value suggested a larger entropy content in the D_2O case owing to increased sampling of macromolecular conformational substates. In contrast, in H_2O , stronger resilience associated with larger fluctuation amplitudes suggested a bigger role of hydration bond interaction in dynamics. The increased flexibility measured in the H_2O case could, however, arise partly from the contribution of exchangeable hydrogens exposed to the solvent, as well as from the differences in viscosity between H_2O and D_2O solvent. The study confirmed the significant role played by the solvent in macromolecular dynamics and provided further evidence of the importance to keep it in mind when replacing H_2O by D_2O for experimental studies.

3.4. Integrated view of intracellular molecular dynamics

The work on *E. coli* water dynamics revealed that the water between the macromolecules in the intracellular environment has essentially bulk-like properties on the

atomic length scale (Jasnin *et al.* 2008a). It was concluded that liquid water flows freely inside cells, within the crowded macromolecular environment. It is very likely that the remarkable properties of bulk water are essential for cellular viability, in particular for functional macromolecular folding, stabilization and activity, transport, membrane formation and protein insertion into membranes. The results demonstrated that intracellular water can participate as such in all the interactions described above. Furthermore, as revealed by the comparison with powder samples, cell water was shown to contribute to internal macromolecular motions on the picosecond time scale (Jasnin *et al.* 2008b). This could be in accordance with the cell water hydrogen-bond network influencing hydrogen-bond dynamics in macromolecular structures. The comparison with dilute protein solutions suggested that weak forces owing to macromolecular crowding may attenuate the lubricating effect of cell water. The results from the solvent isotope effect on macromolecular dynamics in *E. coli* support the presence of water with bulk-like properties in the cell (Jasnin *et al.* 2008c). Replacing H_2O by D_2O has similar effects on cell macromolecules, as has been observed in the dilute solution of BSA (Tehei *et al.* 2001). The lower apparent resilience indicates that the increased stability in D_2O is entropy driven, and results from the increased sampling of conformational substates.

It must be pointed out, however, that the results on protein–water interactions and dynamics strongly depend on the type of organism and its environment. This has been shown by the previous work on halophilic proteins (Tehei *et al.* 2001) and the halophilic organism *H. marismortui* (Tehei *et al.* 2007). In these systems, protein and water dynamics are strongly affected by the molecular interactions with the surrounding salt ions. The highest stability of halophilic proteins in D_2O is enthalpy driven by ion hydration and results in a higher apparent resilience (Tehei *et al.* 2001). A slow water component has been discovered in *H. marismortui*, which is not present in *E. coli*, probably resulting from similar protein–salt ion interactions (Tehei *et al.* 2007).

We concluded that intracellular molecular dynamics is the result of a delicate balance between the structural and dynamical properties of bulk water and macromolecular hydration and interactions.

4. APPLICATION AND PERSPECTIVES

The studies on extremophilic bacteria and *E. coli* revealed that it was possible to get insight into molecular dynamical features in complex systems such as entire cells. *Escherichia coli* has the advantage of being easy to prepare, can be cultivated in fully or partially deuterated medium and can be obtained in the large quantities required for the neutron scattering measurements. *Escherichia coli* was therefore an ideal system for establishing the feasibility of *in vivo* neutron studies. Nevertheless, the work on bacteria constitutes the first step in the *in vivo* neutron studies. It is now essential to go further by exploring different cells and organelles, including eukaryotes and cellular tissue. The

measurements will permit exploring protein dynamics in the presence of cytoskeleton or nucleus or as a function of the cellular state in different organelles. The feasibility of such measurements relies on the appropriate choice of the cell sample. The cells should be easy to prepare in bulk quantities, considering the few hundreds of milligrams of quantities required for the neutron measurements.

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