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Molecular properties of aqueous solutions: a focus on the collective dynamics of hydration water

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When a solute is dissolved in water, their mutual interactions determine the molecular properties of the solute on one hand, and the structure and dynamics of the surrounding water particles (the so-called hydration water) on the other. The very existence of soft matter and its peculiar properties are largely due to the wide variety of possible water–solute interactions. In this context, water is not an inert medium but rather an active component, and hydration water plays a crucial role in determining the structure, stability, dynamics, and function of matter. This review focuses on the collective dynamics of hydration water in terms of retardation with respect to the bulk, and of the number of molecules whose dynamics is perturbed. Since water environments are in a dynamic equilibrium, with molecules continuously exchanging from around the solute towards the bulk and vice versa, we examine the ability of different techniques to measure the water dynamics on the basis of the explored time scales and exchange rates. Special emphasis is given to the collective dynamics probed by extended depolarized light scattering and we discuss whether and to what extent the results obtained in aqueous solutions of small molecules can be extrapolated to the case of large biomacromolecules. In fact, recent experiments performed on solutions of increasing complexity clearly indicate that a reductionist approach is not adequate to describe their collective dynamics. We conclude this review by presenting current ideas that are being developed to describe the dynamics of water interacting with macromolecules.

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1. Introduction

Water permeates our world and its unique capacity of interaction generates a great part of the most amazing forms of matter surrounding us. Many of the peculiar properties of soft matter, including soft biosystems, originate from the structure and dynamics of water, its H-bonding capability and its interaction with solute molecules having different affinities with water. Hydrophilicity and hydrophobicity are multi-faceted phenomena that manifest different characteristics depending on the shape, dimensions and chemical nature of the molecular units,¹ up to the extreme cases of super-hydrophilic² and super-hydrophobic materials.³ Different water affinities are the basis of several processes, from the cleaning action of soaps to the development of microemulsion methods and production of

new materials with tailored wettability, and even the formation, survival and evolution of life as we know it.^{1–4} Moreover, it is well established that water structure and mobility control molecular recognition,^{4–7} and actively participate in the multi-scale motions of proteins,^{8,9} including their collective motions.¹⁰ The role of hydration in the aggregation of misfolded proteins is also important for many diseases, such as prion diseases, diabetes and cancer.¹¹ On a larger scale, water controls the function, quality and stability of more complex matrices, ranging from cells to food and pharmaceutical products;^{12,13} in light of all this, it is impossible to overstate the importance of a thorough knowledge of the properties of water in aqueous solutions. From another perspective, it can be seen that the presence of a solute affects the physical properties of the surrounding water, *e.g.* molecular rotations, density fluctuations and electrical polarization. Partitioning water into two sub-ensembles, hydration and bulk water (Fig. 1), can be seen as the first-level approximation of a more realistic scenario consisting of a continuum of intermediate states with molecules continuously exchanging from around the solute towards the bulk and *vice versa*. The present review focuses on hydration water, especially on how different solute molecules perturb its dynamics and how different experimental techniques are sensitive to such perturbation.

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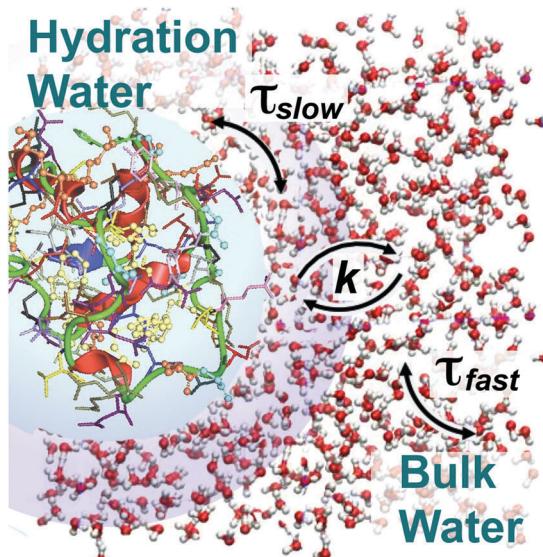


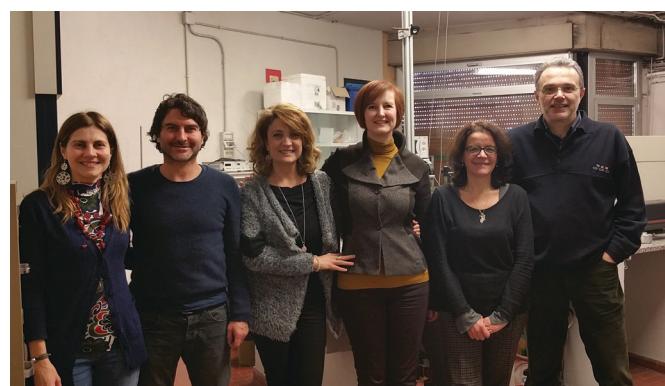
Fig. 1 Hydration is a dynamic process, where bulk and hydration water molecules continuously exchange at a finite rate $k \approx 1/(10\text{--}30 \text{ ps})$.

Several experiments have been performed and many are actively in progress over a wide range of time and length scales, to investigate the static and dynamic properties of hydration water and their relationship with the properties of hydrated molecules. For each experiment, *i.e.* for each physical property being probed, one can define the hydration number N_h as the number of water molecules around the solute having that particular physical property modified with respect to the bulk. Concerning dynamic properties, hydration molecules are usually found to rotate and translate slower than bulk molecules; as a consequence, the second important parameter used to quantify the effect of the solute is the retardation factor $\xi = \tau_{\text{slow}}/\tau_{\text{fast}}$, *i.e.* the ratio between the characteristic relaxation

times of hydration (τ_{slow}) and bulk water (τ_{fast}). The main advantage of analyzing the ratio instead of the absolute τ values is that ξ is less dependent on the kind of correlation functions derived from the experiment (*e.g.* the rank of related Legendre polynomial, in the case of rotational diffusion).

Nevertheless, in recent years, the huge number of experimental and numerical investigations performed on aqueous solutions has produced an increasing number of conflicting values for N_h and ξ , even for experiments performed on the same system. Far from being a problem, we should rather recognize that, in most cases, this is a genuine and informative result: different static and dynamic properties of water surrounding a solute molecule are, indeed, differently perturbed with respect to bulk water. As a consequence, comparing data obtained by different numerical and experimental techniques paves the way for a deeper understanding of the complex behavior of water, and for adding a valuable piece to the puzzle of solvation science.

The number of spectroscopic techniques used to investigate the statics and dynamics of hydration water increases continuously, and providing an exhaustive list is far beyond the scope of this short review. It is worth stressing that the investigation of water behavior may concern either single-particle (self) or collective dynamics. While the self-dynamics has been the subject of extensive theoretical and experimental studies, as reported in recent comprehensive and authoritative reviews,¹⁴ interesting results on collective properties have been only recently obtained with improved experimental techniques. As such, in this review, while referring to different techniques we will place a special emphasis on those revealing collective dynamics. In Section 2 the different techniques are sorted according to the criterion of the probed timescale, which helps clarify the role of the exchanging rate between bulk and hydration water in the possibility of a given spectroscopic



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technique to access the values of both N_h and ξ . Solute concentration effects in diluted solutions will also be considered, in order to distinguish between random close-to-contact and genuine aggregation phenomena. This will also help to reduce ambiguities in comparing results obtained by different experiments.

When trying to compose a complete microscopic picture of water-solute interactions, the reductionist approach is, of course, the most appealing and the first being attempted. Following this approach, the hydration of macromolecules is usually described as the sum of separate contributions arising from the single subunits, which are classified according to their charge distribution and their hydrophilic or hydrophobic attitude. This approach is justified by the relatively high density of cohesive energy in liquid water, which tends to minimize the solute-induced structural changes on the water H-bond network.¹⁵ The same argument is used to suggest that the perturbation should be confined to the first solvation shell. This picture provides a good basis to approach single particle phenomena, such as single particle rotational diffusion of water molecules detected by NMR and molecular dynamics simulations. The state-of-the-art of this approach has been recently reviewed by Fogarty *et al.*¹⁴ However, it has been also recognized that, differently from the single particle behavior, the collective perturbation of water dynamics can extend over a very large distance from the solute (two or more water shells),^{16,17} especially in the case of large and complex solute molecules. In these systems, the collective dynamics of hydration water emerges as a novel feature that cannot be explained as a sum of properties of the solute subunits. Its phenomenology is confirmed by results obtained by different experimental techniques, yet it still needs a full theoretical explanation. Section 3 of the present review, therefore, is focused on the collective dynamics of water around increasingly complex solute molecules, with some recent results of single particle investigations and static (thermodynamic) studies reported for the sake of comparison. Only water-rich solutions are analyzed, which are relevant under physiological conditions and are not affected by additional phenomena arising from water segregation. We will mainly focus on results obtained by extended depolarized light scattering (EDLS),¹⁸ which has been recently proposed by our group as a technique of choice for revealing both hydration numbers and retardation factors in diluted water solutions.

2. Who sees what. Why do different measurements give different values for N_h and ξ ?

There are several criteria to rationalize the values of N_h and ξ obtained by different experiments. One of these has to do with the physical quantities measured by the different techniques, which can be more or less directly linked to the dynamics of water. In fact, some techniques – *e.g.* neutron scattering, NMR, dielectric spectroscopy, light scattering – are directly sensitive to the dynamics of water molecules, while other techniques,

like fluorescence spectroscopy and ESR, make use of external probes and reveal the dynamics of water through its influence on the probe. From another perspective, the experimental techniques could be classified according to the different length and time scales of the probed fluctuations. We will follow this last approach since it properly takes into account the dynamic nature of the solvation process and the crucial role played by the continuous exchange of water molecules from the hydration shell to bulk and *vice versa* (Fig. 1). In fact, within the H-bond network, each water molecule translates by a distance of 0.3 nm, comparable to its diameter, in about 10–30 ps at room temperature,^{19,20} producing a continuous mixing between bulk and hydration molecules. The signal revealed by spectroscopic techniques comes from both water environments, and therefore experimental probes are more or less sensitive to the mixing phenomenon depending on the characteristic times of the explored dynamics. Without referring to any particular theoretical model, we can easily understand that the properties of water molecules “fixed” in their (hydration or bulk) environment are appropriately measured if the probed dynamics is much faster than the exchange between hydration and bulk water (*i.e.* $\tau \ll 10\text{--}30$ ps, or equivalently, frequencies higher than 5–15 GHz). This is the case when the spectroscopic signal arises from processes like intra- and inter-molecular vibrations (bending and stretching modes), librations, the fast structural reorganization of the H-bond network, and high frequency collective dipole reorientational motions. Under these conditions we will have a chance of measuring both N_h and ξ values. Conversely, for water exchange much faster than the probed dynamics (*i.e.* $\tau \gg 10\text{--}30$ ps), as is the case when the spectroscopic signal arises from slow rotational diffusion and conformational changes of solute molecules, only average properties of water can be measured. Under these conditions N_h has to be fixed, for example, to the value derived by geometric arguments, in order to estimate the retardation factor ξ . Finally, the most complicated condition is when the water exchange occurs on a timescale comparable with that of the probed dynamics ($\tau \approx 10\text{--}30$ ps), like for example when the signal is revealing collective dipole relaxation by means of a conventional dielectric setup. In this case, decrypting information from experimental data about relaxation times, diffusion rates and hydration numbers requires a nontrivial model-dependent procedure. These three regimes are analyzed in some detail in the following. In addition, the problem of comparing quasi-static (thermodynamic) with dynamic estimations of N_h is addressed and the effect of the solute concentration briefly analyzed.

2.1 Slow exchange

If the probed dynamics is much faster than the water exchange (frequency higher than 5–15 GHz), bulk and hydration water separately contribute to the spectrum. Few techniques operating in this regime – along with the physical processes they probe – are cited in the following, those which have recently received a remarkable impulse for the study of hydration processes. Among these, some are sensitive to diffusive motions and relaxation processes. Others, like some vibrational spectroscopies,²¹ are more sensitive to the molecular structure, and can reveal the

evolution of the local molecular environment through the fluctuations of the characteristic vibrational frequencies.

Extended depolarized light scattering (EDLS). This technique has recently been proposed as an implementation of depolarized light scattering that, combining dispersive and interferometric setups, gives access to the frequency range from ~ 0.3 to $\sim 3 \times 10^4$ GHz. In this range, both vibrational and relaxational features are revealed, and both ξ and N_h can be estimated. As a typical example, EDLS spectra of pure water and of different water–sugar solutions are shown in Fig. 2.^{22,23} The spectral profile of pure water exhibits two peaks at 1500 and 5100 GHz assigned, respectively, to the H-bond ($O \cdots O \cdots O$ unit) bending and H-bond ($O \cdots O$ unit) stretching vibrations.²⁴ At lower frequencies the water spectrum shows a picosecond relaxation process, centered at about 300 GHz, the structural relaxation of water.^{25,26} In the scope of the present work, this is the most interesting feature since it directly provides the characteristic time of the structural dynamics of water. The major contribution to the scattering of light in this spectral region comes from dipole-induced-dipole (DID) interaction-induced effects, dominated by the collective translational dynamics of water molecules, *i.e.* by the relaxation of the H-bond network.^{27,28} Depolarized light scattering spectra of liquids with low polarizability anisotropy,^{29,30} as in the case of water, have been successfully described considering a collective quantity, the convolution of dynamic structure factors $S(q_0, \omega) \otimes S(q_0, \omega)$, where q_0 is the wavevector corresponding to the maximum of the static structure factor. The picosecond dynamics revealed by EDLS is about 60 times faster than water exchange, so that EDLS spectra can be safely treated under slow-exchange conditions.

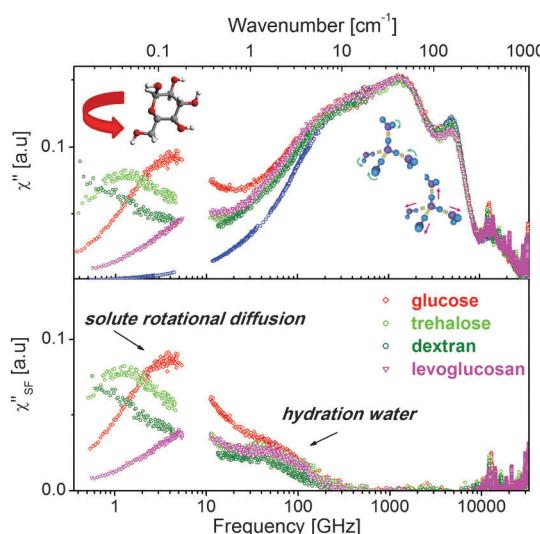


Fig. 2 Depolarized light scattering susceptibility (open circles, upper panel) of water (blue) and different water–sugar solutions at a concentration of 100 mg ml^{-1} and a temperature of 25°C , namely glucose (red), trehalose (green), dextran (dark green) and levoglucosan (purple). The open circles in the lower panel represent solvent-free spectra, obtained by the subtraction of the pure water signal (blue symbols). Two features are well visible: one at frequencies below 10 GHz, which is due to the rotational diffusion of solute molecules, and another around 100 GHz, due to hydration water.

With the addition of the solute, the low frequency part of the spectrum considerably increases due to contributions from hydration water and from diffusion of solute molecules. To highlight solute-induced spectral variations, Fig. 2 shows the solvent-free (SF) spectra, calculated by subtracting the spectrum of pure water from those of the solutions. The feature at around 10 GHz can be assigned to the rotational diffusion of solute molecules, and the other around 100 GHz is due to water molecules whose translational motion is retarded with respect to the bulk. Spectra have been fitted with a Debye function for the solute rotation, two Cole–Davidson functions for hydration and bulk water and two damped harmonic oscillators for bending and stretching modes, as described in ref. 23. The relaxation times τ_{fast} and τ_{slow} and the amplitudes A_{fast} and A_{slow} of bulk and hydration water, determined by the frequency position and area of the relaxation peaks, in the case of negligible cross contributions, give the values of N_h and ξ through the ratios: $N_h = A_{\text{slow}} (A_{\text{slow}} + A_{\text{fast}})^{-1} f^{-1}$ and $\xi = \tau_{\text{slow}} / \tau_{\text{fast}}$, where f is the solute mole fraction. Following this procedure, ξ values ranging from 4 to 8 and a hydration shell thickness from 2 to 9 Å have been found, moving from the simplest to more complex systems, as described in Section 3.

Time resolved optical Kerr effect (OKE). This is a powerful pump–probe technique, with a temporal resolution of femtoseconds, which basically probes, in the time domain, the same physical quantity probed by EDLS experiments. Hence, in principle, both approaches should provide the same dynamic information. Concerning the dynamics of hydration water, several studies have been performed and the changes in the long time portion of the OKE signal induced by a given solute have been rationalized in terms of water relaxation processes.^{31–33}

Quasi-elastic (QENS) and inelastic neutron scattering (INS). These techniques, thanks to the selectivity imposed by the use of protonated and perdeuterated samples, have long contributed to study rotational and translational motions at the picosecond timescale, and vibrational density of states of water surrounding biological molecules, both in solution and in confined geometries, such as in hydrated proteins or peptides.^{34–38} Recently, significant upgrades realized on Brillouin neutron scattering have added valuable knowledge on the fast collective dynamics (high frequency acoustic and optical modes) of biological systems, in the context of biomolecule–solvent mutual interactions.³⁹ Evidence has been provided for perturbed water that belongs to the primary and secondary hydration shells and is less mobile than bulk water.^{40,41} On the other hand, convincing indications have been given that surface protein motions are enhanced by the solvent when their characteristic times are in the picosecond scale, matching those of the breaking and re-formation of hydrogen bonds.^{42–44}

THz spectroscopy. This technique probes the fluctuating orientation of molecular dipoles and the collective intermolecular vibrations of the hydrogen bond network in the THz frequency range.⁴⁵ The THz experiment usually reveals a variation with solute concentration of the absorption at a given single frequency of *ca.* 2.5 THz. Deviations from ideal behavior are attributed to a blue-shift of the vibrational density of states

of hydration water with respect to the bulk, as confirmed by MD simulations. In this frame, the extent of the absorption variation gives the size of the hydration shell, here defined as a “dynamic hydration shell”, involving those water molecules with THz absorbance different from the bulk, or those water molecules characterized by a different (collective) hydrogen bond dynamics at sub-picosecond time scales. Using this technique, hydration shells with a thickness of 3–4 Å in the case of monosaccharides⁴⁶ and up to 15–20 Å in proteins⁴⁵ have been found, suggesting a strong coupling between the solute and solvent vibrational density of states.

Two-dimensional infrared (2D-IR) spectroscopy. This technique tracks water’s time-dependent energetics by monitoring the time correlation function of the transition frequency $\omega(t)$ of the OH stretching mode of dilute HOD in liquid D₂O, whose decay is related to the fast reorganization of the hydrogen bond network.^{21,47} 2D-IR has proven to be able to reveal the solvation dynamics by measuring the characteristic times of exchange reactions, in which the hydrogen bond between a water and a solute molecule switches to another water molecule.^{48,49} Retardation of hydration water molecules with respect to the bulk has also been successfully studied by means of this technique.⁵⁰

Polarization-resolved femtosecond infrared spectroscopy. This technique is used to probe the rotational diffusion of single water molecules, by exciting the uncoupled OH/OD stretching vibration.^{21,50} The rate of molecular reorientation of water molecules is studied by measuring the time dependence of the anisotropy of the excited vibration, by a combination of pump and probe pulses with different polarizations. The main advantage with respect to traditional techniques used for studying single particle rotation, like NMR, is that the full autocorrelation function can be measured. In addition, IR experiments can measure the reorientation of different sub-ensembles through excitation and probe with different photon frequencies.

Raman-MCR. This technique, combining vibrational (Raman) spectroscopy and multivariate curve resolution (MCR),⁵¹ has been recently proposed to derive information on hydration water by revealing subtle solute-induced modifications of the OH stretching Raman signal. The technique has been used to study interactions and aggregation of amphiphilic molecules in water^{52–55} and has revealed the presence of water dangling OH bonds around hydrocarbon groups, contributing to question the traditional view that water forms perfect clathrate–hydrate structures around small hydrocarbon groups.⁵⁶

Femtosecond-resolved fluorescence upconversion technique. It has been extensively used to study the dynamics of hydration water by monitoring the relaxation of the solvation energy of selected cromophores, driven by molecular reorientations and translations.^{5,57} Different from the above-mentioned techniques, fluorescence indirectly reveals the dynamics of water. In fact, it measures the dynamic Stoke shifts of the excited probe (*e.g.* a tryptophan residue in a protein) by monitoring a series of wavelength-resolved fluorescence transients. Two dynamic regimes are usually reported for hydration water: the fast relaxation (1–8 picoseconds, one order of magnitude slower than bulk water) attributed to local water reorientation, and the slow relaxation

(20–200 picoseconds) basically interpreted in terms of dynamic exchange with bulk water. A reinterpretation of this slow relaxation in terms of solvent polarization effects reflecting the protein dynamics has been also proposed.⁵⁸ A recent overview of these fluorescence experiments⁵⁷ recognizes that water and protein motions are coupled, but suggests that the contribution of water prevails over that of the protein to the point that water slaves the protein dynamics.

2.2 Fast exchange

Experimental techniques operating under the fast exchange conditions are those for which the water exchange rate is much faster than the measured relaxation processes. In this limit a single relaxation process is revealed, the average of those of bulk and hydration water. A single measurement cannot discriminate between a moderate slowdown affecting many hydration water molecules and a strongly retarded dynamics affecting few water molecules. To estimate the retardation factor, the value of the hydration number has to be fixed with some independent criterion. Under these conditions operates nuclear magnetic resonance (NMR), a technique used since long time to investigate the reorientation dynamics of individual water molecules at biological interfaces.^{15,59} The number of water molecules affected by a solute molecule is usually estimated through molecular dynamics or solvent-accessible surface area calculations, and typically fixed to a single water layer with a thickness of about 3.5–4 Å. Assuming these values, small retardation factors of about 2 are usually found for the rotational dynamics of hydration water.

2.3 Exchange comparable with the relaxation rate

The most complex situation is that of exchange rate comparable with bulk and hydration relaxation rates. This is the case, for example, of dielectric relaxation spectroscopy, a technique probing the collective dipole orientational relaxation up to tens of picoseconds.^{60–62} In this case, the measured relaxation times and amplitudes are neither those of bulk nor of hydration water but rather a mix of both of them together with the exchange rate. Different phenomenological models have been developed to take into account this phenomenon.^{5,63,64} These models, though not intended to give detailed information at the molecular level, have the merit of giving a key for extracting a more reliable estimation of N_h and ξ from dielectric spectra. As an example, we report in Fig. 3 the relaxation times of water measured in water-TBA mixtures at $T = 5$ °C by dielectric spectroscopy. The spectrum was fitted by two Debye relaxations, with characteristic times τ_1 and τ_2 . If these were directly taken as the relaxation times of hydration and bulk water, one would obtain a retardation τ_1/τ_2 strongly dependent on concentration, varying from 1.8 to 4 in the investigated range. On the other hand, it was shown that the same data can be reasonably reproduced (continuous lines in Fig. 3) by concentration independent relaxation times for bulk ($\tau_{\text{fast}} = 15$ ps) and hydration water ($\tau_{\text{slow}} = 65$ ps), corresponding to a retardation $\xi = 4.3$, and a reasonable value for the exchange time between the two populations of about 38 picoseconds. Nowadays dielectric

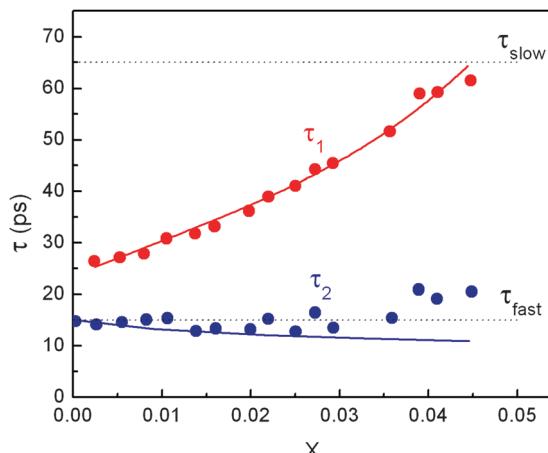


Fig. 3 Dielectric relaxation times, τ_1 and τ_2 , of water-TBA solutions at 5 °C as a function of solute molar fraction.⁶⁰ Full lines are obtained with concentration independent relaxation times for bulk and hydration water, $\tau_{\text{fast}} = 15$ ps and $\tau_{\text{slow}} = 65$ ps, (dotted lines) and an exchange time of 38 picoseconds.

spectroscopy setups are considerably improved and very wide spectral regions can be more easily investigated, up to tens of THz; this allows one to follow the whole diffusive dynamics of both water and solute molecules. Some ‘anomalies’ recently reported in water solutions, such as a small, and concentration dependent-retardation in water-glucose⁶⁵ and water-TMU⁶⁶ solutions might be explained by properly taking into account the exchange of molecules occurring, at a finite rate, between hydration and bulk water.

2.4 Quasi-static (thermodynamics) vs. dynamics

Hydration numbers obtained by quasi-static measurements, like viscosity and compressibility, are typically lower than those deduced by dynamic (spectroscopic) techniques. As an example, the value of N_h obtained from ultrasonic⁶⁷ or viscosity⁶⁸ measurements of water/trehalose solutions is around 12–14 at room temperature, to be compared with $N_h = 24$ –25 obtained by EDLS.²² A possible explanation for this discrepancy can be found in the different aspect of the energy landscape of the solution that determines the structural (thermodynamic) and the dynamic properties of water. In fact, thermodynamic and structural properties are related to the intermolecular interaction energy, *i.e.* to the minima of the energy landscape, and are largely independent of diffusive motions.⁶⁹ Conversely, in the time-scales of interest for hydration phenomena the dynamics is dominated by diffusion and the rates are mainly determined by activation, *i.e.* by the energy of the transition states in the energy landscape.⁶⁹ The larger values of N_h obtained from dynamic measurements with respect to the static ones can be therefore taken as an indication that solute molecules are more effective in perturbing the dynamics of water (activation energies) than its static properties (energy minima). An example of a purely dynamic perturbation is given by the ‘excluded volume’ effect, responsible for retardation of water molecules close to (and ideally not interacting with) solute molecules, as described in ref. 70.

This neat distinction between static and dynamic perturbation effects is also evident from the results of neutron diffraction studies of water-trehalose solutions,⁷¹ which suggest a very minor modification of the water structure, with no more than 3–5 water molecules H-bonded to a single trehalose, while molecular dynamics suggests 13–14 H-bonds and a quite well measurable perturbation of the hydration water dynamics.⁷²

2.5 Concentration effects: aggregation vs. random-close-to-contact

The average number of hydration water molecules per solute molecule, N_h , generally decreases upon increasing solute concentration. Taking this phenomenon into account is of crucial importance, both for a meaningful comparison of results from different experiments, and for a deeper understanding of the aggregation phenomena frequently occurring in water solutions. In this respect, we notice that the value of N_h can decrease with increasing solute concentration also in the case of negligible aggregation, just due to the finite probability of the close-to-contact condition for randomly distributed solute molecules (see the upper panel of Fig. 4). The overlapping of hydration shells gives rise to a release of hydration water towards the bulk. To quantify the contribution of this very basic effect, a simple numerical method based on the

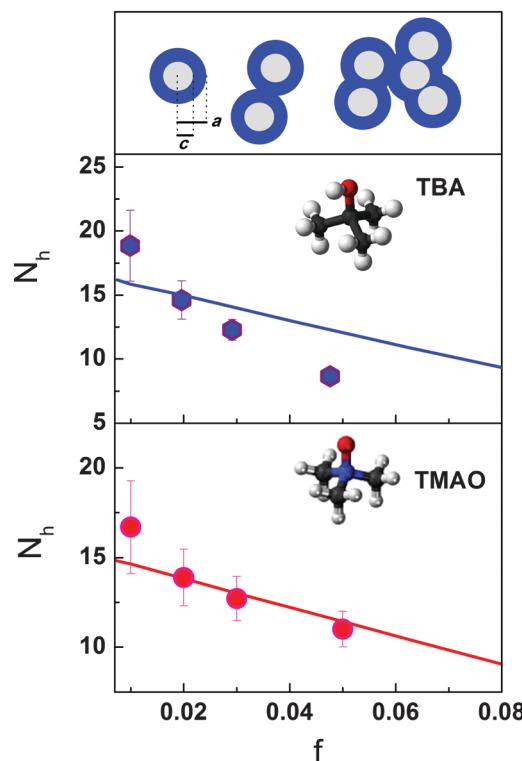


Fig. 4 Average hydration number N_h of TBA and TMAO as a function of molar ratio f . Full lines are calculated supposing that the random-close-to-contact condition between solute molecules (upper panel) is the only mechanism responsible for the reduction of $N_h(f)$. This hypothesis holds quite well in the case of TMAO, while the noticeable deviation of the calculated curve from experimental points in the case of TBA suggests non-negligible aggregation processes to occur in this solution.¹⁹

generation of random distributions of solute-like molecules in water has been recently proposed.^{22,73} Fig. 4 reports the values of N_h measured by EDLS in trimethylamine *N*-oxide (TMAO) and *tert*-butyl alcohol (TBA) water solutions, showing a noticeable reduction of N_h with increasing the solute molar fraction, also under very diluted conditions. An analytical approximation for this model has been proposed for the simplest case of almost-spherical molecules of radius c surrounded by a spherical hydration shell of external radius a . In this case, the maximum hydration number for solute molar ratio $f \rightarrow 0$ is $N_h^0 = 4/3\pi(a^3 - c^3)\rho_H$ where ρ_H is the number density of water. In diluted solutions and in the simple case of dominating two-body effects, the random overlapping of hydration shells gives rise to a reduction of the average hydration number N_h according to the relation $N_h(f) = N_h^0 - \Phi f/(1 + nf)$, where $\Phi = 8/9\rho_H^2\pi^2(a^6 - 8a^3c^3 + 9a^2c^4 - 2c^6)$ and $n = 4/3\pi\rho_Hc^3$.^{23,73} The radius c can be estimated from the rotational diffusion relaxation time derived from the low frequency part of the EDLS spectrum, and the radius a from the value of N_h^0 measured at the lowest concentrations or left as a free fitting parameter. The f dependence of N_h obtained by this procedure is reported in Fig. 4 with solid lines. Here, we can appreciate that in the case of TMAO, the reduction of N_h in the whole concentration range can be attributed to the random-close-to-contact condition. Conversely, in the case of TBA, an additional reduction of N_h occurs, which is the clear signature of aggregation phenomena.¹⁹ A deviation of N_h vs. f from the random-close-to-contact behavior has also been reported in carbohydrate²² and protein⁷⁴ solutions, providing evidence of aggregation processes in those solutions. The importance of a distinction between aggregation and random-close-to-contact conditions has also been evidenced by Raman-MCR studies of water-alcohol solutions.⁵²

In this review, we do not treat extensively the high solute concentration regime. We just recall that nontrivial segregation effects can occur in that case and the dynamics of hydration water can be profoundly affected by the interaction and hindrance of solute molecules.⁷⁵ In this respect, recent UV Raman spectroscopy investigations of hydrophobic portions of solute molecules have suggested a slow-to-fast dynamic transition upon increasing the water content, which could reconcile some contradictory results on retardation factors previously reported in the scientific literature.⁷⁶

3. From small to large: not a simple addition

Soft biological interfaces are a patchwork of hydrophobic, hydrophilic, and charged moieties that interfere with the rearrangement of the H-bond network of water in a very complex way. An important step toward a rationalization is to understand whether and to what extent the results obtained in water solutions of small molecules can be extrapolated to the case of large bio-macromolecules. As anticipated in the Introduction, we focus our attention on the results concerning the dynamic retardation of hydration water and the spatial extent

of such a perturbation, usefully quantified by the values of N_h and ζ . In this regard, a simple approach seems to succeed for the self-dynamics, like the single molecule rotation. Conversely, the collective dynamics probed, for example, by EDLS and THz spectroscopy experiments is found to be affected by the solute molecule much more than what would be expected from the simple addition of effects arising from its small parts. As illustrated schematically in the cartoon of Fig. 7, a significant increase of the water perturbation occurs when increasing the chemical complexity of the solute, suggesting that a reductionist approach is not adequate to describe the collective rearrangement of hydration water. In the following subsections, we review in more detail some results from EDLS in different classes of molecules, in comparison with relevant findings from other techniques.

3.1 Small hydrophobes

Hydrophobic hydration is deeply studied since many years due to its important role in biological processes, ranging from the assembly of lipids in membranes, to protein-ligand binding and protein folding.¹² Small solute molecules are usually selected as simple models to investigate hydrophobic effects.⁵⁶ The influence of hydrophobic groups on the local water structure has suggested the classification of solutes in structure-makers and structure-breakers, a concept still actively debated due to its potential importance in the measured entropy decrease upon hydration of hydrophobic groups.^{56,77–79} The most intriguing concept, however, is that of a hypothetical ice-like cage of immobilized water molecules around each hydrophobic group, proposed by Frank and Evans seventy years ago.⁸⁰ Though the idea of immobilized water molecules has been replaced by that of a retarded diffusion,⁸¹ convincing evidence of a hydrophobically enhanced water structure with greater tetrahedral order has been recently reported on aqueous alcohol solutions by Raman-MCR.⁵⁶ This enhanced structure is found to disappear with increasing temperature and for hydrophobic chains longer than 1 nm, being replaced by a more disordered structure with hydrogen bonds weaker than in bulk water (Fig. 5). These spectroscopic observations are reminiscent of the predicted transition from a more

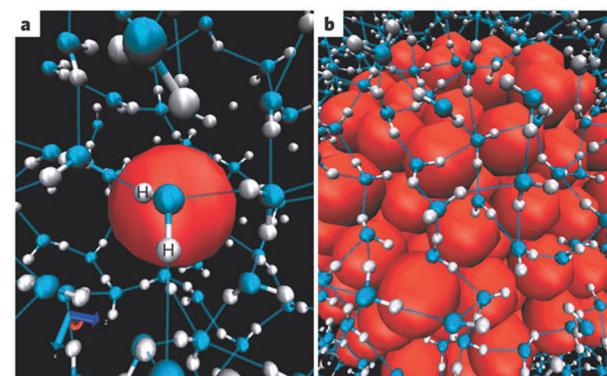


Fig. 5 Configurations of liquid water molecules near hydrophobic cavities in molecular-dynamics simulations. Reprinted with permission from Macmillan Publishers Ltd: [Nature] (ref. 1), copyright (2005).

ordered to a less ordered (air–water-interface-like) hydration-shell structure, with a crossover length scale of the order of 1 nm.¹

EDLS has recently been applied to the study of aqueous solutions of the frequently investigated hydrophobic model systems, *i.e.* TMAO and TBA. These molecules exhibit similar chemical structures having tetrahedral geometry, with three methyl groups as hydrophobic moieties, and a different hydrophilic head, namely a polar N^+O^- and a hydroxyl group, respectively (Fig. 4). Despite the similarities in their structure, these solutes behave quite differently in biological aqueous solutions. The first compound is a potent osmolyte, which is known to enhance the thermodynamic stability of proteins and protect them against denaturation, while the second is a denaturant, showing a destabilizing effect upon the native conformation of proteins. Both in TMAO and TBA solutions, EDLS has clearly identified contributions from both hydration and bulk water, in qualitative agreement with results obtained by OKE experiments³¹ in which, however, only the concentration dependence of the average relaxation time was analyzed, due to intrinsic limitations in the long time (low frequency) regime. In both systems hydration water is found to be dynamically retarded by a factor 4, a value that is smaller than that revealed in a number of different systems, including sugars, peptides, amino acids, and proteins.^{22,28,82–84} The spatial extent of the perturbation induced on surrounding water is limited to the first hydration shell, including about 15–16 molecules, different from more complex amphiphiles, where the perturbation is found to extend up to three or more hydration layers.^{17,18,82,84,85} Overall, these results support the idea that hydration water around small hydrophobic molecules is less perturbed than in all the other cases, a situation very far from that expected in the case of the formation of static ice-like cages.

Indeed, the idea of ice-like cages was introduced to interpret not only the structure but also the time evolution of single particle water diffusion around small hydrophobes measured by femtosecond infrared spectroscopy.⁸¹ An effective immobilization was deduced from the slowing down of few solvating water molecules by a factor not directly measurable due to the restricted time window (<10 ps) probed by the technique. Thereafter, a MD investigation of infrared results suggested that these data could be compatible also with a moderate retardation ($\zeta < 2$) in very diluted solutions, a retardation becoming more and more important with increasing solute concentration.⁷⁰ Another intriguing result of femtosecond infrared spectroscopy was the small number of retarded water molecules, which is about 6–7 for each TMAO and TBA molecule in very diluted solutions. This number is significantly smaller than that obtained by MD simulations, which is 19 and 21, for the two molecules.⁷⁰ Even larger is the geometric hydration number (≈ 25) used to interpret NMR results for TMAO solutions, associated with a very moderate retardation $\zeta \approx 1.9$.⁵⁹ In short, the single-particle water dynamics around small hydrophobic molecules probed by different techniques is actually interpreted either as few (6–7) hydrating particles with large retardation (>4) or as more particles (~ 20) with moderate retardation (<2). Possible explanations have been given either in

terms of a spatially heterogeneous dynamics or in terms of a temporally heterogeneous dynamics of the hydration shell. In the first case “fast” techniques are required since “slow” techniques would give a global average relaxation time;⁸¹ in the second case, especially at high concentration, water confined between solute molecules is described as experiencing a slowly relaxing anisotropic environment.⁵⁹ It is worth noting that for these small hydrophobes the magnitude and extent of the perturbation of the single particle dynamics obtained by the different techniques are, on the average, similar to those of the collective dynamics detected by EDLS.

3.2 Carbohydrates as small hydrophilic model systems

Carbohydrates, together with water, play a fundamental role in many biological processes. The great hydrogen bonding capability together with the high solubility in water makes carbohydrates particularly suitable for the study of the effects induced by hydrophilic interfaces on the properties of water. Moreover, structural and dynamic properties of water–sugar solutions are believed to be responsible for important bioprotective mechanisms.^{86,87} For trehalose, in particular, it has been suggested that its strong bioprotection⁸⁸ may be connected with the effect exerted by the sugar on surrounding water.⁸⁹

EDLS experiments^{22,83,84,90} have been extensively used to gain information on both the number of water molecules dynamically perturbed by mono- and disaccharides and the magnitude of the dynamic perturbation. The retardation induced by glucose, fructose, sucrose and trehalose on the picosecond motions of surrounding water is $\zeta = 5–6$, and is quite independent of the particular system and of concentration. Under most diluted conditions, the effect exerted by each glucose (trehalose) unit extends to 16 (25) water molecules, so that each hydroxyl group slows down the dynamics of about 3.3 solvent molecules. This simple scaling law has been found to hold in a wide concentration range, as shown in Fig. 6 where the hydration number per OH group is reported as a function of hydroxyl groups mole fraction f_h in glucose and trehalose aqueous solutions. This is an emblematic case where the number of perturbed water molecules around the solute can be obtained by the simple addition of molecules perturbed around small solute subunits. A somewhat analogous situation holds for the small hydrophobes TBA and TMAO, which produce a similar perturbation effect. Also in this case, in fact, most of the hydrating water molecules are facing a rather uniform (albeit hydrophobic) interface. For these reasons, hydrophobes and sugars are reported in the low-complexity region of Fig. 7. In this figure, solutes are qualitatively classified in terms of their departure from this simple behavior. According to the current definition, “more is different” in complex systems. Indeed, we will show in the following that the collective dynamics of water around molecules at the right-hand-side of the picture cannot be explained in terms of the hydration properties of their surface subunits. If compared with results obtained for different types of solutes,¹⁷ we notice that sugars are among the least perturbing molecules, with a retardation slightly higher than that of small hydrophobes and a very

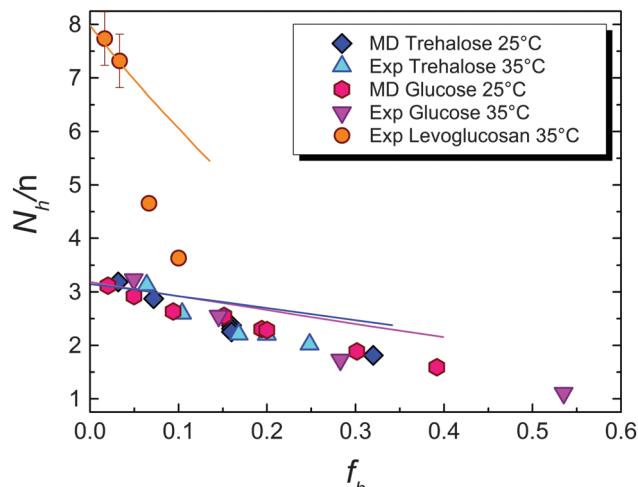


Fig. 6 Trehalose,²² glucose,²³ and levoglucosan²³ aqueous solutions. Normalized hydration numbers N_h/n are reported as a function of the OHs/water mole ratio $f_h = nf$, where n is the number of OHs for each sugar molecule. ($n = 8$ for trehalose, $n = 5$ for glucose, $n = 3$ for levoglucosan). Full lines are the analytical prediction for random close-to-contact solute molecules (see Section 2.5).

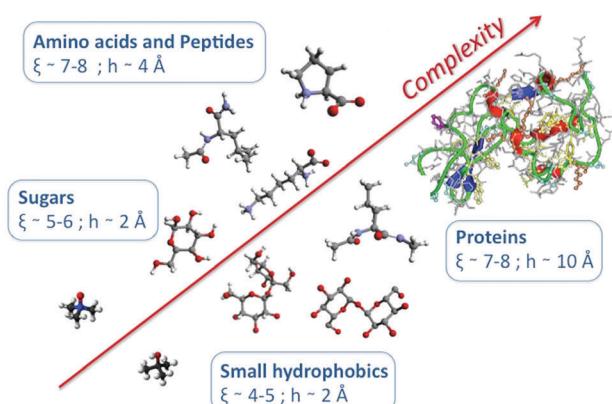


Fig. 7 Schematic representation of the results of recent EDLS experiments on water solutions, showing the retardation of hydration water dynamics ξ with respect to the bulk and the thickness h of the perturbed water layer. A considerable increase in water perturbation is found with increasing complexity in peptides, amino acids, and proteins, with retardation factors up to $\xi = 8$, extending up to three or more water shells. Reprinted from ref. 74, Copyright (2014), with permission from Elsevier.

limited spatial extent, lower than that usually reported for a complete hydration layer, including water molecules at a distance $<3.6\text{ \AA}$ from the oxygen of each hydroxyl group (or $<3.1\text{ \AA}$ from each solute atom).²² This result reinforces the notion that small sugar molecules, with their homogeneously distributed hydroxyl groups, lead to the formation of solute–water hydrogen bonds similar to water–water ones and only to minor changes on the organization of the water network typical of the bulk.⁹¹

The hydration number of sugars obtained by EDLS is intermediate with respect to those obtained by other techniques. In fact, THz absorption experiments reveal a single perturbed hydration shell around glucose.⁴⁶ Conversely, two hydration

layers have been found around disaccharides,⁴⁶ a result that has been the subject of a long debate in the literature.^{92–94} As for single particle rotational diffusion, NMR investigations have suggested a relatively short-range effect (a single hydration shell) with a small dynamic retardation factor of about 1.67.⁹² This estimation is also consistent with results of a very recent polarization-resolved femtosecond infrared study.⁹⁵ “Static” measurements, as expected (see Section 2.4), give smaller hydration numbers. Numeric simulations⁷² and viscosity measurements,⁶⁸ have found an average number of 1.8 water molecules instantaneously H-bonded to each sugar hydroxyl group. Even lower numbers have been found by density measurements,⁹⁶ further evidence that the dynamic perturbation of water around a solute extends toward a larger volume than the static one.

The effects due to the presence and distribution of sugar hydroxyl groups on the dynamics of hydration water have been tested by EDLS for water-levoglucosan (1,6-anhydro- β -D-glucopyranose) (LG) solutions.²³ In fact, LG is different from glucose in that the hydroxymethyl group is blocked, forming an anhydro bridge between 1 and 6 carbon atoms. This small structural change not only reduces from five to three the number of hydroxyl groups, but also produces a drastic reduction in the ability of the molecule to participate in the H-bond network by replacing two H-bond donor/acceptor groups with a single H-bond acceptor site. Indeed, the measured retardation factor ($\xi \approx 3\text{--}4$) is lower than that induced by glucose, suggesting a weaker dynamic perturbation where hydroxyl groups are absent. Conversely, the average number of retarded water molecules around a single levoglucosan molecule is $N_h = 24$, significantly larger than that found for glucose ($N_h = 16$). This is in line with the idea that the disposition of the OH groups on the sugar molecule might favor its insertion within the water H-bond network without strongly perturbing it.⁹¹ Finally, we notice that upon increasing the concentration, specific intermolecular interactions can favor solute aggregation and glucose and trehalose show a similar tendency towards clusterization;²² a stronger effect is observed with levoglucosan, suggesting that larger aggregates are formed in this solution.²³

3.3 Amino acids and peptides

Amino acids and peptides, the building blocks of living matter, are frequently used as model systems to investigate biosystem solvation without the complications arising from the structure and internal dynamics of bio-macromolecules.⁹⁷ An important discontinuity occurs when passing from small hydrophobic and sugar molecules to diluted aqueous solutions of amino acids and dipeptides. In fact, EDLS measurements have shown that, different from the case of small hydrophobes and sugars, both the dynamic slowing down and the spatial extent of the perturbation increase significantly for diluted solutions of amino acids and model dipeptides (NALMA, NALA and NAGMA).^{17,18,85} In particular, a comparison performed at similar concentrations (100 mg ml⁻¹) evidences that the latter compounds induce a dynamic retardation of about 8 that involves up to

ca. 60 surrounding water molecules, and this corresponds to a perturbation that propagates well beyond the first hydration layer. Time-resolved OKE experiments have also revealed a quite large perturbation in the hydration water of different model peptides (NAGMA, NAAMA, NALMA).³² After fixing at 38 the number of perturbed water molecules, dynamic retardation factors ranging from 20 to 12 have been deduced. This result can be easily reconciled with the EDLS one, provided that a smaller retardation is considered for a larger hydration shell.

To notice that these strong effects rely on the different chemical nature of the solutes and not on their size; as a matter of fact the considered amino acids and peptides are even smaller than disaccharides. A long-range influence on water mobility has been recently confirmed in an EDLS study of NALMA at different concentrations, namely a retardation factor ranging from 7 to 9 together with a spatial extent at an infinite dilution of *ca.* 6 Å, encompassing *ca.* 120–150 water molecules.⁸⁵ We also mention that in this case the trend of the hydration number as a function of concentration exhibits a non-linear behavior, highlighting a substantial difference with sugars and small hydrophobes. In this regard, the comparison between the experimental EDLS data and the theoretical solvent-sharing model⁸⁵ has not revealed aggregation phenomena among solute particles up to a concentration approximately equivalent to 40 water molecules per solute, in agreement with the results of small angle scattering measurements used to study the *Q*-dependence of the scattering intensity.⁴⁰

Other spectroscopic techniques sensitive to the collective dynamics of water, such as THz spectroscopy, have also revealed the effects of such a long-range perturbation.⁹⁸ Dielectric studies of water-peptide solutions⁹⁹ have reported anomalies attributed to frustration in the water network arising from the amphiphilic character of the peptide. Actually, difficulties are usually reported for dielectric spectroscopy in separating spectral contributions of bulk and hydration water.¹⁰⁰ As explained in Section 2.3, we suggest that this difficulty may arise from the exchange process, which tends to mix the two relaxation processes.

Neutron scattering experiments have been extensively carried out on peptides. Most of them have been focused on the dynamics of peptides rather than of water, and performed in the very low hydration limit, which is not discussed in the present review. Under more diluted conditions, quasielastic neutron scattering from NALMA-water systems has shown a slowing down of translational dynamics extending to the second hydration shell,⁴⁰ and a weak perturbation of single particle rotational dynamics, confined to the first shell, consistent with NMR results.¹⁵ Complemented by MD simulations, inelastic neutron scattering experiments have also interestingly shown that the density of states of water around hydrophilic and hydrophobic peptides is remarkably similar to that of high density and low density amorphous ice, respectively,¹⁰¹ contributing to the interpretation of the hydration phenomenon in terms of the anomalies of water.

3.4 Proteins

The most striking evidence of the effect of the size and complexity of the solute on the properties of surrounding water has

been found in the case of protein solutions. A recent OKE investigation of lysozyme aqueous solution has reported clear evidence of a spectral component due to hydration water in the range from 10 to 100 GHz.¹⁰² This feature has been studied in more detail by EDLS, revealing a relaxation time of hydration water close to that of amino acids and dipeptides, with a retardation $\xi = 7 \pm 2$.^{74,82} On the other hand, under diluted conditions, the perturbed region around lysozyme has been found to extend upon a considerably wider region of about 12 Å, including over three times the thickness of one geometric hydration shell.⁷⁴ Such a long-range perturbation on surrounding water cannot be rationalized considering a simple combination of effects arising from the independent contribution of single molecular groups on the protein surface. Concerning aggregation phenomena, the presence of lysozyme clusters has been evidenced for concentrations higher than *ca.* 100 mg ml⁻¹. To be observed in these experiments, their lifetimes must be longer than *ca.* 5 ps, the relaxation time of hydration water.

Time-resolved fluorescence spectroscopy, a technique that has pioneered the study of water around proteins,^{5,57,103} has given evidence of coupled water–protein picosecond fluctuations extending beyond the first hydration shell. More recently, THz spectroscopy has proposed a dynamic hydration shell ranging up to 15–20 Å (or even more),⁴⁵ an exceptionally long range effect that has been suggested to be intimately connected with the protein activity, like the control of ice formation by antifreeze proteins¹⁰⁴ and some specific enzymatic activity in human metalloprotease.¹⁰⁵ Interestingly, it has been shown that the coherent collective dynamics of protein hydration water significantly differs from that of neat water, with evidence of a coupling with the dynamics of the protein.¹⁰⁶ A connection has also been found, by neutron scattering, between the translational diffusion of hydration water molecules and the promotion of the large amplitude motions of enzymes required for their biological activity^{106,107} and, by inelastic X-ray scattering, between the presence of hydration water and the softening of collective protein vibrations.¹⁰

As for the single particle rotational dynamics, ¹⁷O NMR has been applied to different proteins and a picture has been proposed,^{15,108} where the dynamics of hydration water is strongly heterogeneous and is modeled by a power-law distribution of rotational relaxation rates. Most of the water molecules in the hydration layer – which is defined *a priori* based on MD simulations and/or surface accessible exposed-area – show a small average retardation factor of about 2 for all the proteins studied (similar to hydration water of small organic solutes). The view of the single particle dynamics derived from these experiments and by MD simulations is depicted in Fig. 8, within the framework of the jump diffusion model for water rotation.^{14,109,110} It is important to emphasize the crucial role recently played by MD simulations for the theoretical understanding of single particle water properties, their relationship with experimental observables,¹¹¹ their peculiar modifications around solutes¹⁴ and their role in determining protein structure and transitions.¹¹² Unfortunately, mainly due to the much heavier computational effort required, only a few examples of

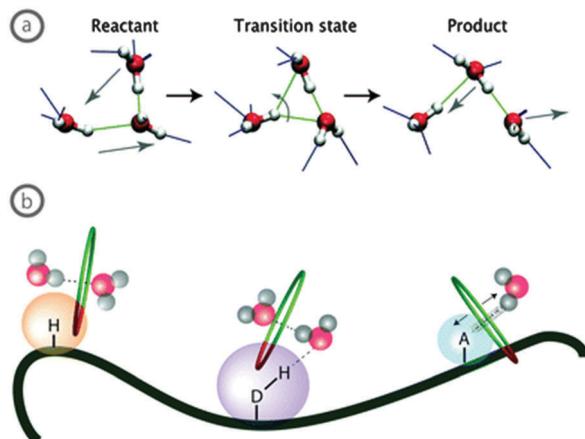


Fig. 8 (a) Molecular jump mechanism proposed for water diffusion in the bulk and (b) at the interface with hydrophobic sites, the H-bond donor and the H-bond acceptor. Reproduced from ref. 14 with permission from the Royal Society of Chemistry.

successful simulations of the collective dynamics probed by EDLS are available like, *e.g.*, those reported for carbohydrate-water solutions.^{22,28} Conversely, in the case of peptide and protein solutions, simulations seem to be still far from reproducing the observed spectroscopic signatures of hydration water.^{113,114} In this respect, we stress that more theoretical investigations would be highly desirable for a deeper understanding of the collective properties of hydration water around large macromolecules.

4. Current challenges and future perspectives

The recent investigation of hydration dynamics has unveiled a very rich reality. The outdated description of hydration water as an ice-like layer surrounding solute molecules has been replaced by a dynamic picture where the hydration water is more or less retarded compared to the bulk. The magnitude of the retardation and its spatial extent is found to depend on the investigated physical quantity, suggesting a multifaceted and not yet completely explored scenario for the solvation processes.

Concerning the single particle rotational diffusion of water, its behavior has been satisfactorily explained by a molecular jump model for the H-bond dynamics,¹¹⁵ extended to include H-bond strength and excluded-volume effects to describe water close to solute macromolecules.¹⁴ Moderate retardation factors, between 1 and 3, are found close to the majority of molecular sites, with larger values limited to buried locations, such as clefts and pockets in proteins.

A richer and more complex phenomenology has been found for the collective dynamics of hydration water. The results of a series of experiments performed on water solutions with solutes of increasing complexity have shown that the influence of a macromolecule on the collective dynamics of surrounding water is not the result of a mere superposition of effects

induced by the individual parts, suggesting that phenomena over different length scales (from atomic to mesoscopic and macroscopic scales) develop different features and require different conceptual approaches.

An effective coupling between water and solute dynamics may be the key for understanding the microscopic origin of some of these “additional” features that appear in the presence of macromolecules, such as peptides and proteins. In fact, their dynamics is characterized by THz modes, which are absent in smaller molecules and which can couple with the dynamics of hydration water. These modes originate from localized motions in peptides and also from collective excitations in larger molecules, like proteins. The collective modes supported by the macromolecules give rise to a well-defined maximum in the density of states in the THz region, the so-called Boson peak. It can be reasonably hypothesized that all these modes can couple with water and, in particular, that collective modes can propagate from the surface through the hydration shell (Fig. 9), as suggested by MD simulations.^{45,106,116} A quite heterogeneous elastic behavior has been depicted for the network of water molecules, whose rigidity increases as the protein surface is approached. Notice that acoustic modes in the THz frequency range are characterized by nanometric wavelengths and that they propagate in the unrelaxed regime, *i.e.* in a solid-like water environment.¹¹⁷ In this regime, acoustic modes are under-damped and can propagate for few wavelengths through surrounding water, the same order of magnitude of the perturbed hydration layer found by EDLS and THz spectroscopies. The low frequency relaxation process measured by EDLS (τ_{slow}) might be the signature of the zero-frequency spectral contribution (Mountain mode) of these THz acoustic modes. The more rigid elastic behavior of hydration water molecules¹⁰⁶ well correlates with their longer relaxation time. In addition, a further coupling channel can be foreseen between protein and water structural dynamics, through intermolecular H-bond stretching modes. In this respect, a schematic mode-coupling

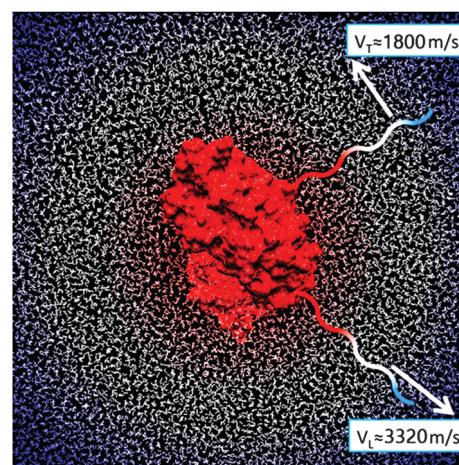


Fig. 9 Illustration of the transverse and longitudinal collective modes propagating in the hydration water of a protein. Reprinted with permission from ref. 45. Copyright (2014) American Chemical Society.

approach has been recently proposed for a complete description of the Raman spectrum, including both the 1.5 THz stretching mode and the low frequency structural relaxation of water.¹¹⁸ In this frame, the coupling of protein internal modes with H-bond stretching modes could be responsible for an enhancement of their spectral contribution. In particular, the observed enhancement of the low frequency relaxation (τ_{slow}) could be connected with a more effective coupling of the solute motions with the low density form of hydration water.

The strong coupling of molecular motions with surrounding water must be bidirectional, involving a comparably strong influence of water on the solute dynamics. The longstanding discussion on who is the master and who is the slave in the water–macromolecule interaction makes us guess that a continuous mutual exchange of energy is, indeed, the reality of the solute–solvent partnership. In this respect, the natural counterpart of the strong and long-range perturbation of collective water dynamics around proteins is a comparably strong effect of water on protein dynamics and functionality, as recently suggested for the case of enzymatic activity. We can thus see the emergence of the need for a deeper understanding of the collective dynamics of hydration water, not only inspired by the pleasure of penetrating the secrets of the most fascinating form of condensed matter. Water, the “matrix of life”, is indeed recognized to play a crucial role in a number of natural and technological processes and a new discipline, “Solvation Science”,¹¹⁹ is gaining ground as an autonomous cross-disciplinary field where chemists, physicists, engineers and biologists cooperate to go beyond the old idea of the solvent as an inert medium, and to unveil its crucial role in solvent-mediated and solvent-controlled processes.

References

- 1 D. Chandler, *Nature*, 2005, **437**, 640–647.
- 2 J. Drelich, E. Chibowski, D. D. Meng and K. Terpilowski, *Soft Matter*, 2011, **7**, 9804.
- 3 M. Callies and D. Quéré, *Soft Matter*, 2005, **1**, 55.
- 4 M. Chaplin, *Nat. Rev. Mol. Cell Biol.*, 2006, **7**, 861–866.
- 5 S. K. Pal and A. H. Zewail, *Chem. Rev.*, 2004, **104**, 2099–2124.
- 6 K. Ariga, T. Nakanishi and J. P. Hill, *Soft Matter*, 2006, **2**, 465–477.
- 7 Y. Levy and J. N. Onuchic, *Annu. Rev. Biophys. Biomol. Struct.*, 2006, **35**, 389–415.
- 8 S. Khodadadi and A. P. Sokolov, *Soft Matter*, 2015, **11**, 4984–4998.
- 9 V. G. Sakai, S. Khodadadi, M. T. Cicerone, J. E. Curtis, A. P. Sokolov and J. H. Roh, *Soft Matter*, 2013, **9**, 5336–5340.
- 10 Z. Wang, W.-S. Chiang, P. Le, E. Fratini, M. Li, A. Alatas, P. Baglioni and S. H. Chen, *Soft Matter*, 2014, **10**, 4298.
- 11 X. Xu, X. Wang, Z. Xiao, Y. Li and Y. Wang, *Soft Matter*, 2012, **8**, 324–336.
- 12 P. Ball, *Chem. Rev.*, 2008, **108**, 74–108.
- 13 A. Cesáro and J. Brady, *Food Biophys.*, 2013, **8**, 151–152.
- 14 A. C. Fogarty, E. Duboué-Dijon, F. Sterpone, J. T. Hynes and D. Laage, *Chem. Soc. Rev.*, 2013, **42**, 5672–5683.
- 15 J. Qvist, E. Persson, C. Mattea and B. Halle, *Faraday Discuss.*, 2009, **141**, 131–144.
- 16 S. Ebbinghaus, S. J. Kim, M. Heyden, X. Yu, U. Heugen, M. Gruebele, D. M. Leitner and M. Havenith, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 20749–20752.
- 17 L. Comez, L. Lupi, A. Morresi, M. Paolantoni, P. Sassi and D. Fioretto, *J. Phys. Chem. Lett.*, 2013, **4**, 1188–1192.
- 18 S. Perticaroli, L. Comez, M. Paolantoni, P. Sassi, A. Morresi and D. Fioretto, *J. Am. Chem. Soc.*, 2011, **133**, 12063–12068.
- 19 L. Comez, M. Paolantoni, L. Lupi, P. Sassi, S. Corezzi, A. Morresi and D. Fioretto, *J. Phys. Chem. B*, 2015, **119**, 9236–9243.
- 20 F. Pizzitutti, M. Marchi, F. Sterpone and P. J. Rossky, *J. Phys. Chem. B*, 2007, **111**, 7584–7590.
- 21 H. J. Bakker and J. L. Skinner, *Chem. Rev.*, 2010, **110**, 1498–1517.
- 22 L. Lupi, L. Comez, M. Paolantoni, S. Perticaroli, P. Sassi, A. Morresi, B. M. Ladanyi and D. Fioretto, *J. Phys. Chem. B*, 2012, **116**, 14760–14767.
- 23 S. Corezzi, P. Sassi, M. Paolantoni, L. Comez, A. Morresi and D. Fioretto, *J. Chem. Phys.*, 2014, **140**, 184505.
- 24 O. Faurskov Nielsen, *Annu. Rep. Prog. Chem., Sect. C: Phys. Chem.*, 1997, **93**, 57–99.
- 25 A. Sokolov, J. Hurst and D. Quitmann, *Phys. Rev. B: Condens. Matter Mater. Phys.*, 1995, **51**, 12865–12868.
- 26 R. Torre, P. Bartolini and R. Righini, *Nature*, 2004, **428**, 296–299.
- 27 M. Paolantoni, P. Sassi, A. Morresi and S. Santini, *J. Chem. Phys.*, 2007, **127**, 024504.
- 28 L. Lupi, L. Comez, M. Paolantoni, D. Fioretto and B. M. Ladanyi, *J. Phys. Chem. B*, 2012, **116**, 7499–7508.
- 29 M. J. Stephen, *Phys. Rev.*, 1969, **187**, 279–285.
- 30 N. J. Tao, G. Li, X. Chen, W. M. Du and H. Z. Cummins, *Phys. Rev. A: At., Mol., Opt. Phys.*, 1991, **44**, 6665–6676.
- 31 K. Mazur, I. A. Heisler and S. R. Meech, *Phys. Chem. Chem. Phys.*, 2012, **14**, 6343–6351.
- 32 K. Mazur, I. A. Heisler and S. R. Meech, *J. Phys. Chem. B*, 2010, **114**, 10684–10691.
- 33 K. Mazur, I. A. Heisler and S. R. Meech, *J. Phys. Chem. A*, 2012, **116**, 2678–2685.
- 34 M. C. Bellissent-Funel, J. Teixeira, K. F. Bradley and S. H. Chen, *J. Phys.*, 1992, **2**, 995–1001.
- 35 A. Frölich, F. Gabel, M. Jasnin, U. Lehnert, D. Oesterhelt, A. M. Stadler, M. Tehei, M. Weik, K. Wood and G. Zaccai, *Faraday Discuss.*, 2009, **141**, 117–130.
- 36 D. Russo, R. K. Murarka, J. R. D. Copley and T. Head-Gordon, *J. Phys. Chem. B*, 2005, **109**, 12966.
- 37 S. Khodadadi, J. H. Roh, A. Kisliuk, E. Mamontov, M. Tyagi, S. A. Woodson, R. M. Briber and A. P. Sokolov, *Biophys. J.*, 2010, **98**, 1321–1326.
- 38 M. Settles and W. Doster, *Faraday Discuss.*, 1996, **103**, 269.
- 39 A. Orecchini, A. Paciaroni, A. D. Francesco, C. Petrillo and F. Sacchetti, *J. Am. Chem. Soc.*, 2009, **131**, 4664–4669.
- 40 D. Russo, G. Hura and T. Head-Gordon, *Biophys. J.*, 2004, **86**, 1852–1862.

- 41 M.-C. Bellissent-Funel, J.-M. Zanotti and S. H. Chen, *Faraday Discuss.*, 1996, **103**, 281.
- 42 A. Orecchini, A. Paciaroni, A. R. Bizzarri and S. Cannistraro, *J. Phys. Chem. B*, 2002, **106**, 7348–7354.
- 43 A. Paciaroni, A. Orecchini, E. Cornicchi, M. Marconi, C. Petrillo, M. Haertlein, M. Moulin, H. Schober, M. Tarek and F. Sacchetti, *Phys. Rev. Lett.*, 2008, **101**, 148104.
- 44 A. Paciaroni, E. Cornicchi, M. Marconi, A. Orecchini, C. Petrillo, M. Haertlein, M. Moulin and F. Sacchetti, *J. R. Soc., Interface*, 2009, **6**, S635–S640.
- 45 V. Conti Nibali and M. Havenith, *J. Am. Chem. Soc.*, 2014, **136**, 12800–12807.
- 46 M. Heyden, E. Bründermann, U. Heugen, G. Niehues, D. M. Leitner and M. Havenith, *J. Am. Chem. Soc.*, 2008, **130**, 5773–5779.
- 47 S. T. Roberts, K. Ramasesha and A. Tokmakoff, *Acc. Chem. Res.*, 2009, **42**, 1239–1249.
- 48 D. Laage and J. T. Hynes, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 967–968.
- 49 A. Ghosh and R. M. Hochstrasser, *Chem. Phys.*, 2011, **390**, 1–13.
- 50 A. A. Bakulin, M. S. Pshenichnikov, H. J. Bakker and C. Petersen, *J. Phys. Chem. A*, 2011, **115**, 1821–1829.
- 51 P. N. Perera, K. R. Fega, C. Lawrence, E. J. Sundstrom, J. Tomlinson-Phillips and D. Ben-Amotz, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 12230–12234.
- 52 D. S. Wilcox, B. M. Rankin and D. Ben-Amotz, *Faraday Discuss.*, 2013, **167**, 177.
- 53 B. M. Rankin, D. Ben-Amotz, S. T. van der Post and H. J. Bakker, *J. Phys. Chem. Lett.*, 2015, **6**, 688–692.
- 54 R. Scheu, B. M. Rankin, Y. Chen, K. C. Jena, D. Ben-Amotz and S. Roke, *Angew. Chem., Int. Ed.*, 2014, **53**, 9560–9563.
- 55 R. Scheu, Y. Chen, H. B. de Aguiar, B. M. Rankin, D. Ben-Amotz and S. Roke, *J. Am. Chem. Soc.*, 2014, **136**, 2040–2047.
- 56 J. G. Davis, K. P. Gierszal, P. Wang and D. Ben-Amotz, *Nature*, 2012, **491**, 582–585.
- 57 D. Zhong, S. K. Pal and A. H. Zewail, *Chem. Phys. Lett.*, 2011, **503**, 1–11.
- 58 B. Halle and L. Nilsson, *J. Phys. Chem. B*, 2009, **113**, 8210–8213.
- 59 J. Qvist and B. Halle, *J. Am. Chem. Soc.*, 2008, **130**, 10345–10353.
- 60 D. Fioretto, A. Marini, M. Massarotti, G. Onori, L. Palmieri, A. Santucci and G. Socino, *J. Chem. Phys.*, 1993, **99**, 8115–8119.
- 61 K. Hallenga, J. R. Grigera and H. J. C. Berendsen, *J. Phys. Chem.*, 1980, **84**, 2381–2390.
- 62 D. Fioretto, A. Marini, G. Onori, L. Palmieri, A. Santucci, G. Socino and L. Verdini, *Chem. Phys. Lett.*, 1992, **196**, 583–587.
- 63 B. Bagchi, *Chem. Rev.*, 2005, **105**, 3197–3219.
- 64 J. E. Anderson, *J. Chem. Phys.*, 1967, **47**, 4879–4883.
- 65 K. Shiraga, T. Suzuki, N. Kondo, T. Tajima, M. Nakamura, H. Togo, A. Hirata, K. Ajito and Y. Ogawa, *J. Chem. Phys.*, 2015, **142**, 234504.
- 66 K.-J. Tielrooij, J. Hunger, R. Buchner, M. Bonn and H. J. Bakker, *J. Am. Chem. Soc.*, 2010, **132**, 15671–15678.
- 67 S. Magazù, P. Migliardo, A. M. Musolino and M. T. Sciortino, *J. Phys. Chem. B*, 1997, **101**, 2348–2351.
- 68 C. Branca, S. Magazù, G. Maisano, F. Migliardo, P. Migliardo and G. Romeo, *J. Phys. Chem. B*, 2001, **105**, 10140–10145.
- 69 B. Halle, *Philos. Trans. R. Soc., B*, 2004, **359**, 1207–1224.
- 70 D. Laage, G. Stirnemann and J. T. Hynes, *J. Phys. Chem. B*, 2009, **113**, 2428–2435.
- 71 S. E. Pagnotta, S. E. McLain, A. K. Soper, F. Bruni and M. A. Ricci, *J. Phys. Chem. B*, 2010, **114**, 4904–4908.
- 72 S. L. Lee, P. G. Debenedetti and J. R. Errington, *J. Chem. Phys.*, 2005, **122**, 204511.
- 73 D. Fioretto, L. Comez, S. Corezzi, M. Paolantoni, P. Sassi and A. Morresi, *Food Biophys.*, 2013, **8**, 177–182.
- 74 S. Perticaroli, L. Comez, P. Sassi, M. Paolantoni, S. Corezzi, S. Caponi, A. Morresi and D. Fioretto, *J. Non-Cryst. Solids*, 2015, **407**, 472–477.
- 75 K. L. Ngai, S. Capaccioli and A. Paciaroni, *Chem. Phys.*, 2013, **424**, 37–44.
- 76 F. D'Amico, B. Rossi, G. Camisasca, F. Bencivenga, A. Gessini, E. Principi, R. Cucini and C. Masciovecchio, *Phys. Chem. Chem. Phys.*, 2015, **17**, 10987–10992.
- 77 P. Buchanan, N. Aldiwan, A. K. Soper, J. L. Creek and C. A. Koh, *Chem. Phys. Lett.*, 2005, **415**, 89–93.
- 78 K. A. Sharp and J. M. Vanderkooi, *Acc. Chem. Res.*, 2010, **43**, 231–239.
- 79 N. Galamba, *J. Phys. Chem. B*, 2013, **117**, 2153–2159.
- 80 H. S. Frank and M. W. Evans, *J. Chem. Phys.*, 1945, **13**, 507–532.
- 81 Y. L. A. Rezus and H. J. Bakker, *Phys. Rev. Lett.*, 2007, **99**, 148301.
- 82 S. Perticaroli, L. Comez, M. Paolantoni, P. Sassi, L. Lupi, D. Fioretto, A. Paciaroni and A. Morresi, *J. Phys. Chem. B*, 2010, **114**, 8262–8269.
- 83 M. Paolantoni, L. Comez, M. E. Gallina, P. Sassi, F. Scarponi, D. Fioretto and A. Morresi, *J. Phys. Chem. B*, 2009, **113**, 7874–7878.
- 84 S. Perticaroli, M. Nakanishi, E. Pashkovski and A. P. Sokolov, *J. Phys. Chem. B*, 2013, **117**, 7729–7736.
- 85 L. Comez, S. Perticaroli, M. Paolantoni, P. Sassi, S. Corezzi, A. Morresi and D. Fioretto, *Phys. Chem. Chem. Phys.*, 2014, **16**, 12433.
- 86 J. H. Crowe, L. M. Crowe and D. Chapman, *Science*, 1984, **223**, 701–703.
- 87 D. Corradini, E. G. Strekalova, H. E. Stanley and P. Gallo, *Sci. Rep.*, 2013, **3**, 1218.
- 88 A. Cesàro, *Nat. Mater.*, 2006, **5**, 593–594.
- 89 S. Magazù, V. Villari, P. Migliardo, G. Maisano and M. T. F. Telling, *J. Phys. Chem. B*, 2001, **105**, 1851–1855.
- 90 M. Paolantoni, L. Comez, D. Fioretto, M. E. Gallina, A. Morresi, P. Sassi and F. Scarponi, *J. Raman Spectrosc.*, 2008, **39**, 238–243.
- 91 P. E. Mason, G. W. Neilson, J. E. Enderby, M. L. Saboungi and J. W. Brady, *J. Phys. Chem. B*, 2005, **109**, 13104–13111.

- 92 L. R. Winther, J. Qvist and B. Halle, *J. Phys. Chem. B*, 2012, **116**, 9196–9207.
- 93 M. Heyden, G. Schwaab and M. Havenith, *J. Phys. Chem. B*, 2014, **118**, 10802–10805.
- 94 B. Halle, *J. Phys. Chem. B*, 2014, **118**, 10806–10812.
- 95 C. C. M. Groot and H. J. Bakker, *Phys. Chem. Chem. Phys.*, 2015, **17**, 8449–8458.
- 96 A. Gharsallaoui, B. Rogé, J. Génotelle and M. Mathlouthi, 4th International Workshop on Water in Foods, 2008, vol. 106, pp. 1443–1453.
- 97 C. Malardier-Jugroot, M. E. Johnson, R. K. Murarkawb and T. Head-Gordon, *Phys. Chem. Chem. Phys.*, 2008, **10**, 4903–4908.
- 98 B. Born, H. Weingärtner, E. Bründermann and M. Havenith, *J. Am. Chem. Soc.*, 2009, **131**, 3752–3755.
- 99 R. K. Murarka and T. Head-Gordon, *J. Phys. Chem. B*, 2008, **112**, 179–186.
- 100 P. Sasisanker and H. Weingärtner, *ChemPhysChem*, 2008, **9**, 2802–2808.
- 101 D. Russo, J. Teixeira, L. Kneller, J. R. D. Copley, J. Ollivier, S. Perticaroli, E. Pellegrini and M. A. Gonzalez, *J. Am. Chem. Soc.*, 2011, **133**, 4882–4888.
- 102 D. A. Turton, H. M. Senn, T. Harwood, A. J. Lapthorn, E. M. Ellis and K. Wynne, *Nat. Commun.*, 2014, **5**, 3999.
- 103 T. Li, A. A. Hassanali, Y.-T. Kao, D. Zhong and S. J. Singer, *J. Am. Chem. Soc.*, 2007, **129**, 3376–3382.
- 104 K. Meister, S. Ebbinghaus, Y. Xu, J. G. Duman, A. DeVries, M. Gruebele, D. M. Leitner and M. Havenith, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 1617–1622.
- 105 M. Grossman, B. Born, M. Heyden, D. Tworowski, G. B. Fields, I. Sagi and M. Havenith, *Nat. Struct. Mol. Biol.*, 2011, **18**, 1102–1108.
- 106 V. Conti Nibali, G. D'Angelo, A. Paciaroni, D. J. Tobias and M. Tarek, *J. Phys. Chem. Lett.*, 2014, **5**, 1181–1186.
- 107 G. Schirò, Y. Fichou, F.-X. Gallat, K. Wood, F. Gabel, M. Moulin, M. Härtlein, M. Heyden, J.-P. Colletier, A. Orechini, A. Paciaroni, J. Wuttke, D. J. Tobias and M. Weik, *Nat. Commun.*, 2015, **6**, 6490.
- 108 C. Mattea, J. Qvist and B. Halle, *Biophys. J.*, 2008, **95**, 2951–2963.
- 109 F. Sterpone, G. Stirnemann and D. Laage, *J. Am. Chem. Soc.*, 2012, **134**, 4116–4119.
- 110 A. C. Fogarty and D. Laage, *J. Phys. Chem. B*, 2014, **118**, 7715–7729.
- 111 C. Calero, J. Martí and E. Guàrdia, *J. Phys. Chem. B*, 2015, **119**, 1966–1973.
- 112 V. Bianco and G. Franzese, *Phys. Rev. Lett.*, 2015, **115**, 108101.
- 113 D. R. Martin, D. Fioretto and D. V. Matyushov, *J. Chem. Phys.*, 2014, **140**, 035101.
- 114 D. R. Martin and D. V. Matyushov, *J. Chem. Phys.*, 2014, **141**, 22D501.
- 115 D. Laage, G. Stirnemann, F. Sterpone, R. Rey and J. T. Hynes, *Annu. Rev. Phys. Chem.*, 2011, **62**, 395–416.
- 116 M. Heyden and D. J. Tobias, *Phys. Rev. Lett.*, 2013, **111**, 218101.
- 117 A. Cunsolo, G. Ruocco, F. Sette, C. Masciovecchio, A. Mermet, G. Monaco, M. Sampoli and R. Verbeni, *Phys. Rev. Lett.*, 1999, **82**, 2810.
- 118 A. Taschin, P. Bartolini, R. Eramo, R. Righini and R. Torre, *Nat. Commun.*, 2013, **4**, 2401.
- 119 K. Morgenstern, D. Marx, M. Havenith and M. Muhler, *Phys. Chem. Chem. Phys.*, 2015, **17**, 8295–8296.