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Two structural relaxations in protein hydration water and their dynamic crossovers

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We study the translational single particle dynamics of hydration water of lysozyme upon cooling by means of molecular dynamics simulations. We find that water close to the protein exhibits two distinct relaxations. By characterizing their behavior upon cooling, we are able to assign the first relaxation to the structural α-relaxation also present in bulk water and in other glass-forming liquids. The second, slower, relaxation can be ascribed to a dynamic coupling of hydration water motions to the fluctuations of the protein structure. Both relaxation times exhibit crossovers in the behavior upon cooling. For the α-process, we find upon cooling a crossover from a fragile behavior to a strong behavior at a temperature which is about five degrees higher than that of bulk water. The *long*-relaxation time appears strictly connected to the protein motion as it shows upon cooling a temperature crossover from a strong behavior with a lower activation energy to a strong behavior with a higher activation energy. The crossover temperature coincides with the temperature of the protein dynamical transition. These findings can help experimentalists to disentangle the different information coming from total correlators and to better characterize hydration water relaxations in different biomolecules. *Published by AIP Publishing.* [http://dx.doi.org/10.1063/1.4959286]

I. INTRODUCTION

The role of hydration water in biological systems has been the subject of many studies.[1–8,81] Water stabilizes the three-dimensional structure of a protein and mediates interactions between biomolecules. When a minimum level of hydration is not reached, proteins lack biological function. Water offers indeed a wide range of conformational hydrated states to proteins which are not present in their crystal phases and promotes exchanges between those states that are necessary for the biological processes. Therefore solvent water is essential for the functioning of proteins.[9]

Water near biological surfaces is perturbed with respect to its bulk phase both in the structure and in the dynamics.[6,7,10–12] Depolarized light scattering experiments[10,13,14] detected two distinct relaxations when measuring spectra of aqueous solutions of biomolecules. The first one is a bulk-like relaxation while the second one is 6-8 time slower than the first one and this second relaxation was specifically ascribed to hydration water. Simulations on aqueous solutions of water and biomolecules upon cooling[15,16] have also detected these two kinds of relaxations with the same retardation factor. The correlators analyzed in these studies were total correlators where a part of the contribution to its shape comes from hydration water and the other part comes from the bulk-like water contained in the solution. Femtosecond resolved fluorescence experiments[17,18] explicitly showed that at ambient temperature, there are two relaxation times coming from the solvation shell. By using biological probes, Zhang *et al.*[19] also found that at ambient temperature, the solvation process always occurs through two relaxations which can differ up to one order of magnitude. The slower relaxation appears due to the coupling with the protein motions.[19–21] Therefore several experiments show that hydration water appears not only slowed down by its interaction with the biosurface but also "bimodal" in the dynamics.

In the supercooled regime an important temperature characterizes the so-called protein dynamical transition (PDT), which is a sharp increase in the protein flexibility occurring upon increasing temperature. This transition is observed by neutron scattering experiments,[22–24] as well as by simulation works[23,25–28] and terahertz dielectric response experiment.[29] It is believed to be driven by the hydration water mainly because of the absence of this transition in dry protein samples.

In a seminal paper, Chen *et al.*[22] studied the single particle translational dynamics of a monolayer coverage of water close to lysozyme by quasielastic neutron scattering upon cooling. They found a single slow structural relaxation time upon cooling for low Q exchanged wave-vectors, up to $Q = 1.1$ Å^{-1}, and found that this relaxation is the analogous of the $\alpha-$ relaxation of bulk water, a kind of relaxation typical of glass formers. This relaxation shows a fragile to strong transition close to the PDT. These findings were confirmed by further experiments and simulations and further studies on the diffusion coefficient.[23,27,28]

The considerable effort done by the community in this field lead to substantial progresses in the understanding of

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hydration water slow translational dynamics, nonetheless the picture is still not complete mainly because of the fact that experiments and simulations often access only to a part or to an average of the complete microscopic picture that we would ideally like to have.

In this work, we use molecular dynamics (MD) simulations to study an aqueous solution of a globular protein, a lysozyme. With a high statistic analysis of the MD trajectories, we calculated the density correlators coming only from hydration water at the peak of the oxygen structure factor, $Q = 2.25$ Å^{-1} for which the features of the slow dynamics upon cooling are best evident,[30] and we found two translational structural relaxations in this water. We will show in the following that the first slow relaxation is the analogous of the α-relaxation of bulk water, therefore the relaxation detected by Chen *et al.*[22] and successive papers. The second slow relaxation that we find, occurring over a longer time scale is peculiar only of hydration water and it is more difficult to detect at lower Q values.

Having calculated the behavior of the two slow relaxations that we find, as a function of temperature upon cooling, we detect two distinct dynamic crossovers, one for each relaxation time.

Importantly we underline, before starting to present our results, that we do not treat rotational dynamics here; therefore, results with techniques that measure rotational relaxations (like dielectric relaxation spectroscopies) cannot be directly compared with our results. In particular, the fragile to strong transitions as predicted by Mode Coupling Theory (MCT) are strongly linked to translational dynamics as a decoupling between rotational and translational degrees of freedom happens at supercooled temperatures when the cage of the first neighbors starts to relax (at the α-relaxation time). A detailed study on this decoupling in bulk water is reported in Ref. 31.

In Sec. II, we describe the system and the computational details. Sec. III deals with the density correlators and the fits done to get the two slow relaxation times. Sec. IV deals with the α-relaxation, and Sec. V deals with the slowest relaxation and its relation with the PDT. Sec. VI is devoted to conclusions.

II. SYSTEM AND COMPUTATIONAL METHODS

We performed MD all-atoms simulations on the lysozyme globular protein immersed in water.

Our system is composed of 1 lysozyme protein, 13 982 water molecules, and 8 Cl^- ions to maintain the total charge neutrality. Protein bonded and non-bonded interactions were modeled by the CHARMM force field[82,83] and water was modeled by the SPC/E potential.[84] The cutoff radius for the non-bonded van der Waals interactions was set to 10 Å. Particle mesh Ewald method was used to handle the electrostatic interactions. Verlet leap-frog algorithm, with a time step of 1 fs, was used to integrate the equations of motion. We performed MD simulations using GROMACS 4.5.5[85] package. We simulated the system at constant pressure $p = 1$ bar and at ten different temperatures $T = 300$ K, $T = 280$ K, $T = 260$ K, $T = 250$ K, $T = 240$ K, $T = 230$ K, $T = 220$ K, $T = 210$ K, $T = 205$ K, and $T = 200$ K. Both temperature and pressure were handled with the Berendsen method.[86] Equilibration times range from a minimum of 30 ns for higher temperatures to 100 ns for lower temperatures. At each temperature, the equilibration run was followed by a variable production run of 20 ns (high T) or 30 ns (low T) to calculate dynamic quantities as density correlators. The total computational time required on a single cpu for this study would be ~10 yr.

In this work, we focused our analysis on the protein hydration water, namely, water molecules at the protein interface. Typically hydration water can be defined in a shell of 4-6 Å[6,7,15,16] around macromolecules. In the present work, we will refer as hydration water of lysozyme as water molecules at a distance minor or equal to 6 Å from any lysozyme atoms. In Figure 1, we show a 20 Å slice cut projected in two dimensions of our system where the hydration water defined by the above criterion is highlighted.

We characterized the translational dynamics of hydration water by calculating the oxygen spatial Fourier transform of the single particle density-density correlator also known as the Self-Intermediate Scattering Function (SISF),

$$F_{\mathrm{self}}^{\mathrm{OO}}(Q,t) = \frac{1}{N}\left\langle \sum_{i=1}^{N} e^{i\vec{Q}\cdot[\vec{r}_i(t)-\vec{r}_i(0)]}, \right\rangle \quad (1)$$

where N is the number of water molecules, $\vec{r}_i(t)$ is the position of the oxygen atom of the i-th water molecule at time t, and $\vec{Q}$ the transferred wave-vector. All the SISFs here reported are calculated at a transferred wave-vector $Q_{max} = |\vec{Q}_{max}| = 2.25$ Å^{-1}, namely, at the first peak of the oxygen-oxygen structure factor of water. At this wave-vector,

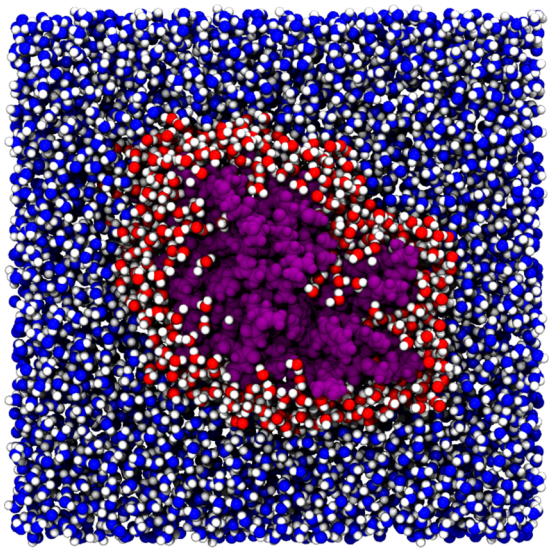

FIG. 1. Snapshot of the simulated lysozyme in water at $T = 300$ K. The image corresponds to a 20 Å slice in the z direction projected into the xy plane of the 75 Å × 75 Å × 75 Å simulation box. The lysozyme is depicted as purple spheres. Water molecules are colored according to their distance from the lysozyme: red (oxygens) and white (hydrogens) molecules correspond to hydration water, namely, water molecules within a distance up to 6 Å from the protein atoms. The rest of the water molecules are shown in blue (oxygens) and white (hydrogens).

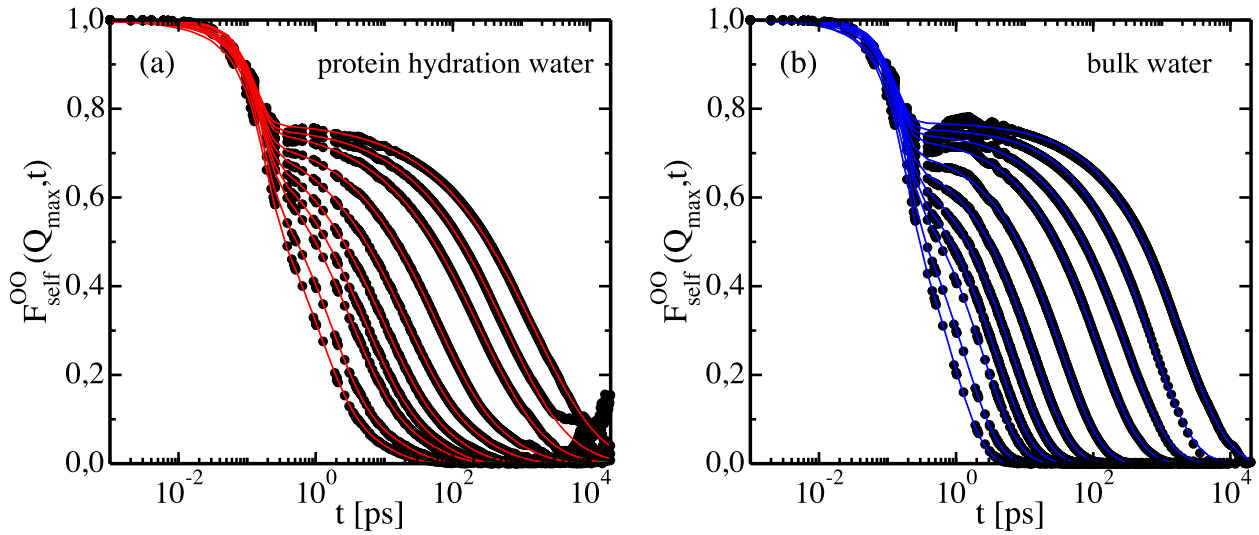

FIG. 2. Self-intermediate scattering function (SISF) of the oxygen atoms of lysozyme hydration water (panel (a)) and bulk water (panel (b)). SISFs (black filled circles) are calculated at the peak of the oxygen-oxygen structure factor $Q_{max} = 2.25$ Å^{-1} from $T = 300$ K (bottom curve) to 200 K for the water lysozyme system (top red curve) and down to 195 K for the bulk system (top blue curve). Continuous lines superimposed to the data are the best fit obtained using Eq. (2) (blue) for bulk water and Eq. (3) (red) for hydration water. The parameters of the fit can be found in the supplementary material.[32]

the features of the slow dynamics upon cooling are best evident as it corresponds to the cage of the first neighbors.[30]

The SISFs of hydration water are computed for oxygen atoms of water molecules inside the 6 Å shell around the lysozyme and only for the time they reside in the shell. In order to gain enough statistics for the correlators of particles moving in such a small space, we analyzed the trajectories of all 13 982 oxygen atoms of the systems to achieve the best possible statistics from our MD trajectories for the long time tails of the correlators.

For comparing the results on hydration water with pure bulk water, we also simulated a reference system composed of 500 SPC/E water molecules at the same pressure of the water and lysozyme system and for the same temperatures plus T = 195 K. For SISFs of bulk water, we computed the quantity in Eq. (1) from MD trajectories by considering all water molecules of the simulation box.

III. DENSITY-DENSITY CORRELATORS

We analyzed hydration water surrounding our globular protein considering a 6 Å-thick shell around the protein. The long relaxation that we calculated in this region extends on ns time scales and in this lapse of time, the single particle visits both hydrophilic and hydrophobic sites. This type of analysis has the averaging character of several experimental techniques that are not sensible to site-specific interactions, like quasielastic neutron scattering.

Figure 2 shows hydration water SISFs and bulk water SISFs calculated from our trajectories at the peak of the oxygen-oxygen structure factor for temperatures ranging from 300 K down to 200 K for hydration water and down to 195 K for bulk water.

We observe for both systems, the development upon cooling of a two step relaxation scenario typical of glass formers with the correlators that become more and more stretched as we lower the temperature. In Figure 3, we also compare the computed SISFs for hydration water in the water-lysozyme aqueous solution and for bulk water at selected temperature. Here the global slowing down of the dynamics of hydration water with respect to the bulk is clearly visible. At a given temperature, the correlator of hydration water always decays to zero on a longer time scale with respect to bulk water. This effect becomes more pronounced at low temperatures. At 200 K, the correlator of hydration water is still not zero after 20 ns, while the bulk correlator at the same temperature decays at about 3 ns. Besides, the SISFs coming from hydration water have a different shape and the diversification with respect to the bulk correlators is more evident at long times.

It is known that, upon cooling, the dynamics of bulk water is well described by the Mode Coupling Theory (MCT) of glassy dynamics.[30,33–35] In this theory, the motion of the tagged particle occurs on two different time scales, a short time during which, after an initial ballistic motion, the particle

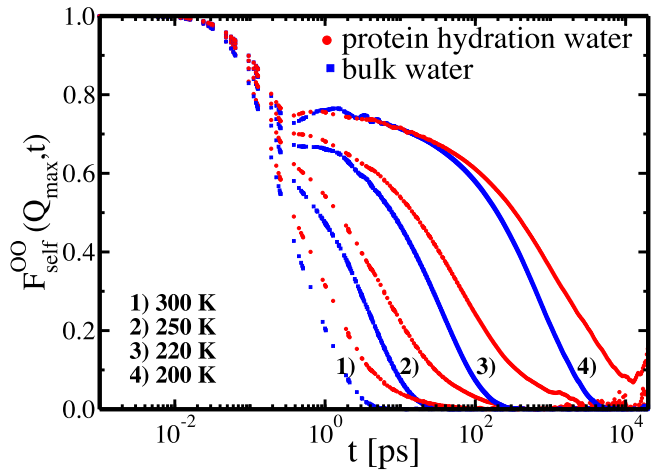

FIG. 3. Comparison of SISFs of bulk water (blue) and hydration water of the water and lysozyme system (red) at selected temperatures.

is trapped within the cage formed by its nearest neighbors, and a longer time during which the cage relaxes and the particle is free to move away and eventually restores the Brownian behavior. The relaxation time connected with this relaxing cage is the α-relaxation time, a typical feature of glass formers.

The shape of the SISF of supercooled water was modeled according to MCT by Gallo *et al.*[33] and Sciortino *et al.*[34] by the sum of a Gaussian function with time constant τ_{short} that takes into account the initial ballistic motion, and a stretched exponential function, known as the Kohlrausch-Williams-Watts function, for the structural α-relaxation, with time constant τ_α and a stretching parameter β_α,

$$F_{\text{self}}^{\text{OO}}(Q,t) = (1 - f_Q)\exp\left[-\left(\frac{t}{\tau_{\text{short}}}\right)^2\right] + f_Q \exp\left[-\left(\frac{t}{\tau_\alpha}\right)^{\beta_\alpha}\right]. \quad (2)$$

Our bulk water SISFs fit Eq. (2) (see Figure 2(b)) as expected. We find instead that the SISFs of hydration water do not fit this model. In particular, the onset of long time tails in hydration water cannot be taken into account by this equation.

When studying the dynamics of water in presence of disaccharides in solution, Magno and Gallo[15] observed a behavior similar to that of our solution on the total correlator and modified Eq. (2) by adding a second stretched exponential function to take into account a second relaxation not present in bulk water. With this modification, the SISF takes the form,

$$F_{\text{self}}^{\text{OO}}(Q,t) = (1 - f_Q - f'_Q)\exp\left[-\left(\frac{t}{\tau_{\text{short}}}\right)^2\right] + f_Q \exp\left[-\left(\frac{t}{\tau_\alpha}\right)^{\beta_\alpha}\right] + f'_Q \exp\left[-\left(\frac{t}{\tau_{\text{long}}}\right)^{\beta_{\text{long}}}\right], \quad (3)$$

where τ_{long} and β_{long} are, respectively, the relaxation time and the stretching parameter of the *long*-relaxation.

We find here that the SISFs of hydration water fit very well this last model, see Figure 2(a). The complete set of fitting parameters of the SISFs can be found in the supplementary material.[32]

While in previous studies on water and biomolecules performed in our group[15,16] and in several experiments[10,13,14] it had been impossible to distinguish whether the α-relaxation came only from those water molecules not in direct contact with the biomolecule (bulk-like water) or also from the hydration layer, with the high statistics reached in the present study, we can now assert that the translational relaxation of hydration water is not a simply bulk-like relaxation with a longer time constant. Hydration water relaxes through two distinct processes, an α-relaxation and a *long*-relaxation, occurring on two different time scales. The process labeled as *long* arises only for water close to the protein.

The two relaxation times of hydration water extracted from the fit together with their respective stretching parameters are reported as a function of temperature in Fig. 4. In the same figure, bulk water values are also reported. The structural relaxation time of hydration water, τ_α, shares the same time

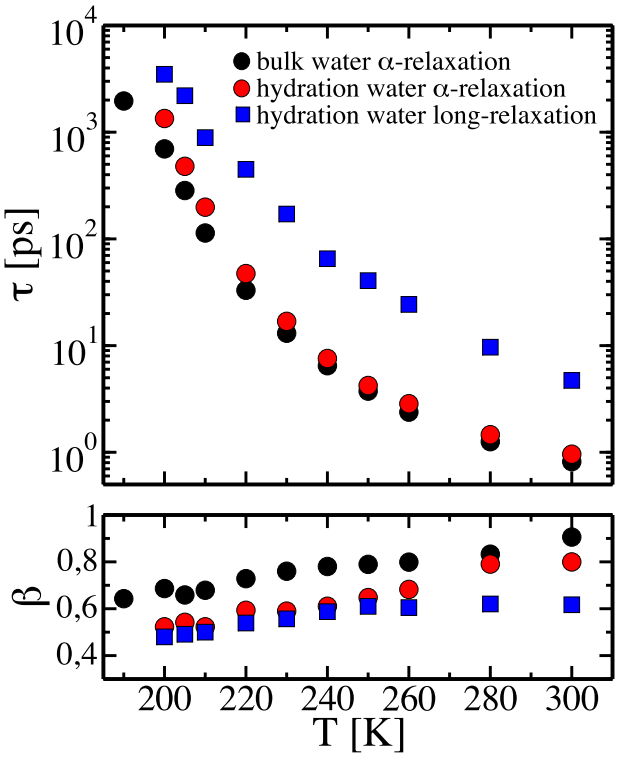

FIG. 4. Upper panel: structural relaxation times, $\tau_\alpha^{\text{bulk}}$ (black filled circles), τ_α (red filled circles), τ_{long} (blue filled squares). Lower panel: stretching parameters $\beta_\alpha^{\text{bulk}}$, β_α, β_{long} (same symbols) as a function of temperature, extracted from the fit procedure as described in the text.

scale of the structural α-relaxation time of bulk water $\tau_\alpha^{\text{bulk}}$, i.e., tenth to hundred of picoseconds. Moreover, they display the same temperature behavior upon cooling, showing similar values apart from a slight slowing down of hydration water more evident at the lowest temperatures. The longer relaxation process, not present in bulk water, occurs over a longer time scale, from tens to thousands of picoseconds and the relaxation time τ_{long} shows a different temperature behavior.

IV. THE α-RELAXATION AND THE FRAGILE-TO-STRONG TRANSITION

We first discuss the α-relaxation of bulk and hydration water. The fact that the major diversification in the dynamics of hydration water comes from the existence of the *long* relaxation is already evident from the direct comparison of the bulk and hydration water correlators in Figure 3. Especially for the lowest temperature, it is clear that the α-relaxation of hydration water is similar to the bulk. The two curves indeed almost coincide for two decades from the plateau of the correlators and only after circa 100 ps the hydration water correlators begin to stretch showing long tails.

From Figure 4, we see that at a given temperature, the structural relaxation of hydration water is more stretched than that of bulk water, since $\beta_\alpha < \beta_\alpha^{\text{bulk}}$, but they show the same temperature trend. In particular, upon cooling, the α-relaxation becomes more stretched, highlighting the increasing departure from an exponential decay, until a constant value is reached at the lowest temperatures. The same temperature trends and

similar values have been found for the stretching parameters of confined water.[36,37]

In Figures 5(a) and 5(b), we show the structural relaxation times of bulk and hydration water. The bulk structural relaxation time τ_α follows the MCT predictions and in the region of mild supercooling, its temperature behavior is described by a power law $\tau_\alpha \sim (T - T_C)^{-\gamma}$, where T_C is the MCT temperature which marks the ideal transition from an ergodic to a non-ergodic phase. According to the ideal version of MCT below T_C, the SISF no longer decays to zero and the dynamics is arrested. This transition is "ideal" because in most structural glass formers hopping phenomena, also

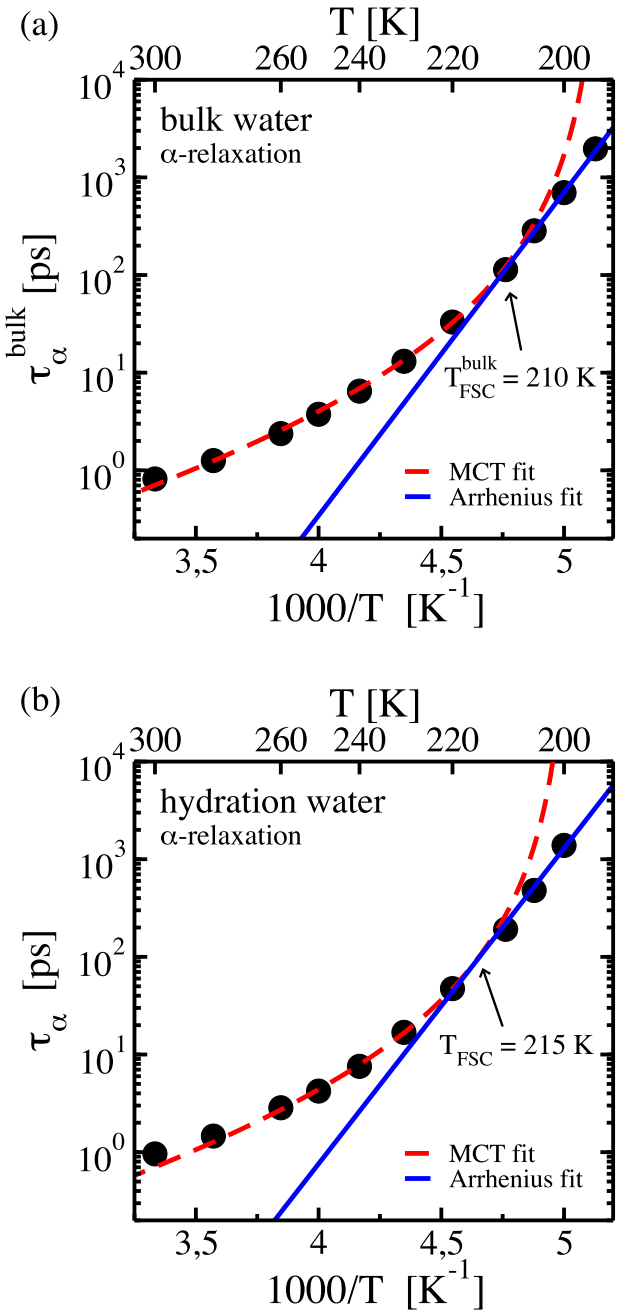

FIG. 5. Arrhenius plots of the α-relaxation times τ_α^{bulk} in bulk water (panel (a)) and τ_α in lysozyme hydration water (panel (b)). The points fit the MCT power law at high temperatures and the Arrhenius law at low temperatures. See Table I for fit parameters of the two regimes.

TABLE I. Top part: MCT temperature (T_C), γ exponent, activation energy (E_A), and FSC temperature (T_{FSC}) for the α-relaxation of bulk SPC/E water and SPC/E water of hydration of lysozyme. Bottom part: activation energies (E_{A_1}), (E_{A_2}), and SSC temperature (T_{SSC}) for the *long*-relaxation of SPC/E water of hydration of lysozyme.

	T_C (K)	γ	E_A (kJ/mol)	T_{FSC} (K)
Bulk water				
τ_α	193.8	2.74	63.4	210
Hydration water				
τ_α	199.0	2.68	61.9	215
	E_{A_1} (kJ/mol)		E_{A_2} (kJ/mol)	T_{SSC} (K)
Hydration water				
τ_{long}	26.6		39.3	240

called activated phenomena, restore ergodicity and the SISF continue decaying. In line with this behavior, as reported in Fig. 5, we find that τ_α^{bulk} of bulk water can be fitted with the MCT power law only excluding lower temperatures points. The fit gives an MCT temperature $T_C^{bulk} = 193.8$ K and a power exponent $\gamma = 2.74$, in agreement with previous studies on the bulk phase.[33,34] The lower temperature points behavior can be instead described by the Arrhenius law $\tau_\alpha \sim e^{E_A/(K_B T)}$ that is commonly used for glass formers and describes well the region of hopping/activated processes. Therefore a crossover from a fragile, power-law, regime to a strong, hopping dominated, Arrhenius regime occurs. We found the fragile-to-strong crossover (FSC) in SPC/E bulk water to occur at a $T_{FSC}^{bulk} = 210$ K at 1 bar isobar, with an activation energy $E_A^{bulk} = 63.4$ kJ/mol. The same value was reported in Ref. 38 as extracted from the FSC of the diffusion coefficient $D \sim \tau_\alpha^{-1}$.

We now perform this MCT test on the τ_α of protein hydration water. We find that, despite the moderate slowing-down induced by the protein, the α-relaxation time of hydration water is not dramatically influenced by the protein and retains the same phenomenology of bulk water. In particular, it follows the MCT predicted power law with an MCT temperature $T_C = 199.0$ K and a power exponent similar to the bulk $\gamma = 2.68$. We observe the deviation from the MCT prediction at $T_{FSC} = 215$ K, when the FSC takes place and below this crossover hydration water can be described as a strong liquid with a slightly lower activation energy respect to the bulk of $E_A = 61.9$ kJ/mol (Figure 5). Fit parameters are reported in Table I.

The phase diagram of bulk supercooled water is very complex, see for example Refs. 39–44. The FSC of the structural α-relaxation in water has been related to the crossing of a liquid-liquid Widom line[45–53] (WL), the line of collapse of the maxima in response functions that converge to a liquid-liquid critical point (LLCP).[39,40,54] Therefore the FSC that we found could be the trace of the presence of a WL and consequently of a LLCP in the phase diagram of protein hydration water. This possibility has been discussed, for example, in Refs. 28, 55, and 87. In particular, the Widom line separates a high density liquid phase region from a low density one, where the less dense liquid favors the hopping processes and therefore the strong behavior of water. The

similar value of activation energy of bulk and hydration water supports the idea that the leading dynamic process in the region below T_{FSC}^{bulk} and T_{FSC} is the same. Below T_{FSC}^{bulk} where hopping processes dominate, the activation energy has been linked to the energy of hydrogen bonds (see, for example, Ref. 56 and references therein). Gillen, Douglass, and Hoch[57] reported experimental activation energies from the self-diffusion coefficient in water in the broad range (242–473) K. The activation energy is increasing upon cooling water. At 242 K, E_A^{bulk} is ~46 kJ/mol, this value approximately corresponds to the energy required to break four hydrogen bonds at that temperature. Recently Dehaoui, Issenmann, and Caupin[58] reported $E_A^{bulk} \sim 53$ kJ/mol for the low temperature activation of the viscous flow, even if they also show a decoupling between viscosity and diffusion coefficient at low temperature in water. It appears that in supercooled experimental water, E_A^{bulk} is increasing upon decreasing temperature toward the value reported in ice Ih ($E_A^{Ih} \sim 61$ kJ/mol).[59] We note that the ice value is close to the values that we found for both SPC/E bulk and hydration water, as extracted from our low temperature ($T < 220$ K) behavior of τ_α. This limit has not so far been probed in experiments due to the limitations of freezing. In the strong regime, water has been shown to be below the Widom line and therefore on the low density side of the liquid.[45,49,53] The low density liquid is characterized by a more open and locally tetrahedral structure.[60,61] An open interesting hypothesis is that in supercooled water, the hydrogen bond network is more energetic than in ice.[62] We also note that both T_C and T_{FSC} are about five degrees shifted to higher temperature in protein hydration water with respect to the bulk water values. The shift of water dynamics to higher temperatures has also been observed for many systems including sugar-water solutions,[15,16] electrolytes solution,[63–65] and confined water.[36,66,67] This indicates the possibility that exploring the phase diagram of hydration water and finding a shifted LLCP may overcome experimental issues related to the crystallization of bulk water.[68]

Coming back to Figure 2, we also observe that the overshoots of the density correlators at the beginning of the plateau are present both in the bulk and in hydration water for the lowest temperatures. The first bump is a manifestation of a low frequency scattering excess, also known as Boson peak, present in bulk, confined, and biological water.[69–72] Recently the onset of the Boson peak in water has been also connected to the presence of a liquid-liquid WL both in bulk and confined water.[69,73–76] The oscillating features above 1 ps are not present for hydration water since they are finite size effects that disappear for larger box sizes, as discussed in detail by Kumar *et al.*[69]

In summary, the performed MCT test on the τ_α of protein hydration water in our system establishes that the correspondent process is an α-relaxation typical of glass formers and it is analogous to that of bulk water. As stated in the Introduction, the α-relaxation and the FSC in hydration water was also found on a slightly different system: a lysozyme protein with a monolayer coverage of water both in experiments and in simulations.[22,27,77] We must however say that the water monolayer coverage in simulation and the water monolayer coverage in the powders of the experiments[22,27,77] could introduce differences in dynamics with respect to our hydration water in a solution. Besides, at the Q values investigated in these works (less or equal to $Q = 1.1$ Å^{-1}), the two slow relaxations have different weights in the correlators with respect to the ones that we have at the first peak of the oxygen-oxygen structure factor and might not be distinguishable.

V. THE LONG RELAXATION AND THE PROTEIN DYNAMICAL TRANSITION

Now we discuss the *long*-relaxation of hydration water. The relaxation time τ_{long} extracted from the fit of SISFs via Eq. (3) is reported in Figure 6. It is evident from the figure that two distinct dynamic regimes, linear on the Arrhenius plot, occur. We fit them both with the Arrhenius law $\tau_{long} \sim e^{E_A/(K_B T)}$ obtaining two different activation energies. A lower one for the high temperature regime $E_{A_1} = 26.6$ kJ/mol and a higher one for the low temperature regime $E_{A_2} = 39.3$ kJ/mol. This strong-to-strong crossover (SSC) appears at about $T_{SSC} = 240$ K. Fit parameters are reported in Table I.

The stretching exponent β_{long} of this relaxation, see Fig. 4, is almost constant at the highest temperatures, and has a slight decrease for the last temperatures starting at about $T_{SSC} = 240$ K following the crossover of its relaxation time τ_{long}. At high temperature, the *long*-relaxation appears globally more stretched than the α process, while below 240 K they reach similar values.

Given also the experimental evidence discussed in the introduction,[10,13,17–19] we ascribe the existence of this *long*-relaxation time and its behavior to the coupling of hydration water with protein structure fluctuations. In particular, we infer that long time rearrangements of water molecules dragged

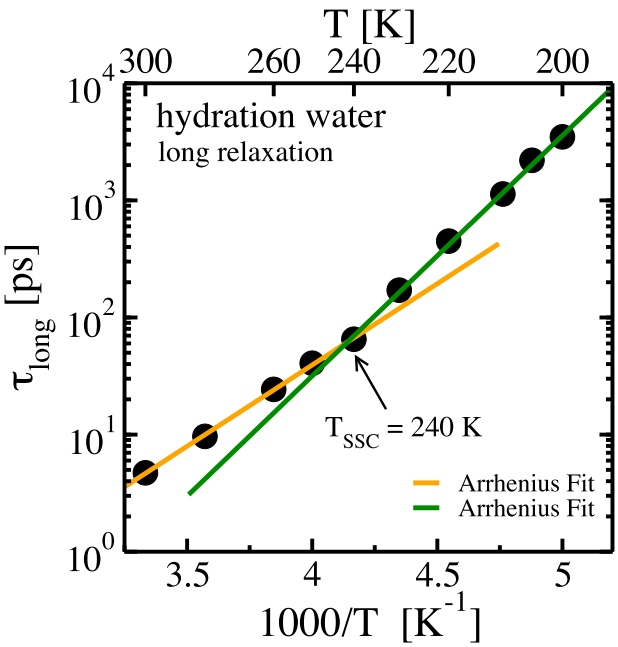

FIG. 6. Arrhenius plot of the *long*-relaxation time τ_{long} of hydration water. The points fit Arrhenius laws with different activation energies at high (E_{A_1}) and low (E_{A_2}) temperatures. All the fit parameters and crossover temperatures between different regimes are reported in Table I.

by the protein cause the onset of the *long*-relaxation of hydration water. When the protein moves, water molecules in the hydration shell have to reorganize according to the protein motion. Since protein motions like rotations of side chains at surface (10-10^2 ps at room temperature) or relative motions of globular domains (10-10^5 ps) happen on long time scales, typically much longer than that of water network relaxation, the water network can relax for the α process independently, which is what we observed in the present study.

In other words, due to the different time scales, water molecules see the protein at fixed position and can relax and diffuse as in bulk-like environment (α relaxation and FSC crossover to hopping), while only over long times the water network follows the protein changes of shape (*long*-relaxation and SSC). The onset of this second, longer relaxation in hydration water is therefore strictly connected to the fluctuations in the protein structures.

To test our interpretation, we calculate the mean square displacement (MSD) Δr^2 of hydrogen atoms of lysozyme. We choose hydrogen atoms because they are the lightest and the most mobile over the surface of the protein and therefore a useful probe for the mobility of the protein surface.

We compute the hydrogen MSD at each time step by comparing the protein structure at time t with respect to the reference initial structure of the protein through a least-squares fitting of the two compared structures. This procedure completely removes the translational motion of the protein during the simulation and therefore the MSD can be considered as measure of fluctuations in the structure of the protein itself. The time dependent MSD is then averaged over 1 ns. Figure 7 displays the final protein MSD Δr^2 as a function of temperature for our system.

According to Ref. 78, the MSD of a protein is made of two contributions, a vibrational component that dominates at low temperatures and that, apart from the zero-point constant value, is linear with the temperature. And a conformational component that is temperature activated and absent when the protein is dry. We clearly observe a dynamic transition in the protein MSD at around T = 240 K. The MSD is weakly dependent on temperature below 240 K. At $T = 240$ K, its slope changes and increases. This steep increase means that the protein structure fluctuations are enhanced above 240 K and this activates the functioning of the protein. This transition is the well-known protein dynamical transition.[79] The PDT is linked to changes in the conformational states of the protein and it was observed both in neutron scattering experiments[22–24,80] and in simulations.[23,25–28] Importantly, various studies showed that the PDT occurs for small globular proteins always between 220 K and 240 K quite independently of the water potential used, see for example Refs. 25 and 26 for SPC/E, Refs. 23 and 27 for TIP4P-Ew, Ref. 28 for TIP5P.

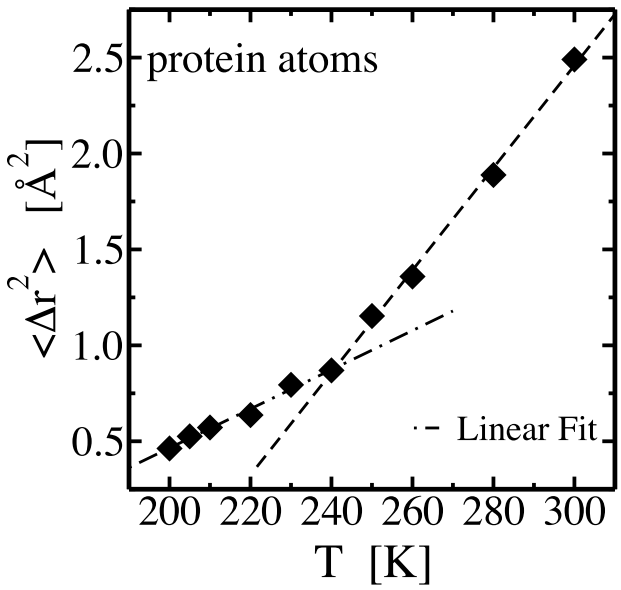

FIG. 7. MSD of protein hydrogen atoms as a function of temperature. Each point represents the average over the last nanosecond of the collected MD trajectory. The MSD exhibits a crossover at $T = 240$ K. Dashed lines are best fit lines done to determine the crossover location.

Around $T = 240$ K, we showed that also the *long*-relaxation has a crossover (SSC) and this proves that the two phenomena are linked. It is worth noting that the more the protein fluctuates, the faster the *long*-relaxation of hydration water is. In that respect the crossing to a lower activation energy upon increasing temperature is a consequence for hydration water of the coupling with the faster protein fluctuations at high temperatures.

VI. SUMMARY AND CONCLUSIONS

In summary, we have shown that the translational dynamics of water proximal to protein shows two well distinct slow relaxation processes. The first one, happening on a faster time scale, is the α structural relaxation typical of glass formers and in particular of bulk water. This relaxation in water is due to the continuous breaking and reforming of hydrogen bonds. The structural relaxation time of the α process exhibits a fragile to strong crossover upon cooling, which might be associated with the crossing of the Widom line as it happens in the bulk phase. The second relaxation, absent in bulk water, happens on a longer time scale and appears to be the macroscopic effect of the dynamic coupling between hydration water and the protein. It exhibits a change in the temperature trend at 240 K and this temperature corresponds to an enhancement in the mean square displacement of protein atoms, the PDT. Based on our result, we conclude that the complex thermodynamics of supercooled water plays a fundamental role only in the α-relaxation, while it seems that the *long*-relaxation is completely driven by the protein motion.

Our characterization of hydration water relaxation times can help experimentalists to interpret the information coming from total correlators in order to better characterize hydration water relaxations in different proteins and possibly biomolecules. We in fact recall here that the considerations that we did on hydration water of lysozyme in our previous study based on the analysis of the total correlators,[16] namely, the existence of a long relaxation that disappears if hydration water is excluded from the correlator analysis, are analogous to those done for aqueous solutions of water and trehalose and water and maltose.[15] So, based on our very small statistics (only trehalose, maltose, and lysozyme), the long-relaxation might

be always present in hydration water whatever biomolecule is in the aqueous solution, at least down to a certain extension of the biomolecule, that of disaccharides (molecular weight circa 350).

[1]L. Cordone, G. Cottone, A. Cupane, A. Emanuele, S. Giuffrida, and M. Levantino, Curr. Org. Chem. **19**, 1684 (2015).
[2]E. Mamontov and X.-q. Chu, Phys. Chem. Chem. Phys. **14**, 11573 (2012).
[3]G. Cottone, J. Phys. Chem. B **111**, 3563 (2007).
[4]P. W. Fenimore, H. Frauenfelder, B. H. McMahon, and F. G. Parak, Proc. Natl. Acad. Sci. U. S. A. **99**, 16047 (2002).
[5]H. Frauenfelder, P. Fenimore, and B. McMahon, Biophys. Chem. **98**, 35 (2002), special issue in Honour of Maurizio Brunori.
[6]A. R. Bizzarri and S. Cannistraro, J. Phys. Chem. B **106**, 6617 (2002).
[7]C. Rocchi, A. R. Bizzarri, and S. Cannistraro, Phys. Rev. E **57**, 3315 (1998).
[8]J. A. Rupley and G. Careri, Adv. Protein Chem. **41**, 37 (1991).
[9]F. Franks, *Water: A Matrix of Life*, 2nd ed., RSC Paperbacks (The Royal Society of Chemistry, Cambridge, UK, 2000).
[10]S. Perticaroli, L. Comez, P. Sassi, M. Paolantoni, S. Corezzi, S. Caponi, A. Morresi, and D. Fioretto, J. Non-Cryst. Solids **407**, 472 (2015).
[11]S. Perticaroli, D. Russo, M. Paolantoni, M. A. Gonzalez, P. Sassi, J. D. Nickels, G. Ehlers, L. Comez, E. Pellegrini, D. Fioretto, and A. Morresi, Phys. Chem. Chem. Phys. **17**, 11423 (2015).
[12]R. d. C. Barbosa and M. C. Barbosa, Physica A **439**, 48 (2015).
[13]L. Comez, L. Lupi, A. Morresi, M. Paolantoni, P. Sassi, and D. Fioretto, J. Phys. Chem. Lett. **4**, 1188 (2013).
[14]S. Corezzi, P. Sassi, M. Paolantoni, L. Comez, A. Morresi, and D. Fioretto, J. Chem. Phys. **140**, 184505 (2014).
[15]A. Magno and P. Gallo, J. Phys. Chem. Lett. **2**, 977 (2011).
[16]D. Corradini, E. G. Strekalova, H. E. Stanley, and P. Gallo, Sci. Rep. **3**, 1218 (2013).
[17]S. K. Pal, J. Peon, B. Bagchi, and A. H. Zewail, J. Phys. Chem. B **106**, 12376 (2002).
[18]S. K. Pal, J. Peon, and A. H. Zewail, Proc. Natl. Acad. Sci. U. S. A. **99**, 1763 (2002).
[19]L. Zhang, L. Wang, Y.-T. Kao, W. Qiu, Y. Yang, O. Okobiah, and D. Zhong, Proc. Natl. Acad. Sci. U. S. A. **104**, 18461 (2007).
[20]T. Li, A. A. Hassanali, Y.-T. Kao, D. Zhong, and S. J. Singer, J. Am. Chem. Soc. **129**, 3376 (2007).
[21]A. Paciaroni, E. Cornicchi, M. Marconi, A. Orecchini, C. Petrillo, M. Haertlein, M. Moulin, and F. Sacchetti, J. R. Soc. Interface **6** (Suppl. 5), S635 (2009).
[22]S.-H. Chen, L. Liu, E. Fratini, P. Baglioni, A. Faraone, E. Mamontov, and M. Fomina, Proc. Natl. Acad. Sci. U. S. A. **103**, 9012 (2006).
[23]S.-H. Chen, M. Lagi, X.-q. Chu, Y. Zhang, C. Kim, A. Faraone, E. Fratini, and P. Baglioni, Spectrosc. Int. J. **24**, 1 (2010).
[24]G. Schirò, M. Fomina, and A. Cupane, J. Chem. Phys. **139**, 121102 (2013).
[25]K. Wood, A. Frölich, A. Paciaroni, M. Moulin, M. Härtlein, G. Zaccai, D. J. Tobias, and M. Weik, J. Am. Chem. Soc. **130**, 4586 (2008).
[26]G. Schirò, Y. Fichou, F.-X. Gallat, K. Wood, F. Gabel, M. Moulin, M. Härtlein, M. Heyden, J.-P. Colletier, A. Orecchini, A. Paciaroni, J. Wuttke, D. J. Tobias, and M. Weik, Nat. Commun. **6**, 6490 (2015).
[27]M. Lagi, X.-q. Chu, C. Kim, F. Mallamace, P. Baglioni, and S.-H. Chen, J. Phys. Chem. B **112**, 1571 (2008).
[28]P. Kumar, Z. Yan, L. Xu, M. G. Mazza, S. V. Buldyrev, S.-H. Chen, S. Sastry, and H. E. Stanley, Phys. Rev. Lett. **97**, 177802 (2006).
[29]A. G. Markelz, J. R. Knab, J. Y. Chen, and Y. He, Chem. Phys. Lett. **442**, 413 (2007).
[30]W. Götze, *Complex Dynamics of Glass-Forming Liquids: A Mode-Coupling Theory* (Oxford University Press, New York, USA, 2009).
[31]S.-H. Chen, P. Gallo, F. Sciortino, and P. Tartaglia, Phys. Rev. E **56**, 4231 (1997).
[32]See supplementary material at http://dx.doi.org/10.1063/1.4959286 for the set of fitting parameters of hydration and bulk water SISFs (Table T1).
[33]P. Gallo, F. Sciortino, P. Tartaglia, and S.-H. Chen, Phys. Rev. Lett. **76**, 2730 (1996).
[34]F. Sciortino, P. Gallo, P. Tartaglia, and S.-H. Chen, Phys. Rev. E **54**, 6331 (1996).
[35]R. Torre, P. Bartolini, and R. Righini, Nature **428**, 296 (2004).
[36]P. Gallo, M. Rovere, and S.-H. Chen, J. Phys. Chem. Lett. **1**, 729 (2010).
[37]P. Gallo, M. Rovere, and S.-H. Chen, J. Phys.: Condens. Matter **24**, 064109 (2012).
[38]F. W. Starr, F. Sciortino, and H. E. Stanley, Phys. Rev. E **60**, 6757 (1999).
[39]P. H. Poole, F. Sciortino, U. Essmann, and H. E. Stanley, Nature **360**, 324 (1992).
[40]O. Mishima and H. E. Stanley, Nature **396**, 329 (1998).
[41]P. G. Debenedetti, J. Phys.: Condens. Matter **15**, R1669 (2003).
[42]J. R. Errington and P. G. Debenedetti, Nature **409**, 318 (2001).
[43]K. Ito, C. T. Moynihan, and C. A. Angell, Nature **398**, 492 (1999).
[44]P. Gallo, K. Amman-Winkel, C. A. Angell, M. A. Anisimov, F. Caupin, C. Chakravarty, E. Lascaris, T. Loerting, A. Panagiotopoulos, J. Russo, J. Sellberg, H. E. Stanley, H. Tanaka, C. Vega, L. Xu, and L. G. M. Pettersson, Chem. Rev. **116**, 7463 (2016).
[45]L. Xu, P. Kumar, S. V. Buldyrev, S.-H. Chen, P. H. Poole, F. Sciortino, and H. E. Stanley, Proc. Natl. Acad. Sci. U. S. A. **102**, 16558 (2005).
[46]L. Liu, S.-H. Chen, A. Faraone, C.-W. Yen, and C.-Y. Mou, Phys. Rev. Lett. **95**, 117802 (2005).
[47]G. Franzese and H. E. Stanley, J. Phys.: Condens. Matter **19**, 205126 (2007).
[48]J. L. F. Abascal and C. Vega, J. Chem. Phys. **133**, 234502 (2010).
[49]P. Gallo and M. Rovere, J. Chem. Phys. **137**, 164503 (2012).
[50]D. Corradini, P. Gallo, S. V. Buldyrev, and H. E. Stanley, Phys. Rev. E **85**, 051503 (2012).
[51]E. Salcedo, N. M. Barraz, and M. C. Barbosa, J. Chem. Phys. **138**, 164502 (2013).
[52]J. Luo, L. Xu, E. Lascaris, H. E. Stanley, and S. V. Buldyrev, Phys. Rev. Lett. **112**, 135701 (2014).
[53]M. De Marzio, G. Camisasca, M. Rovere, and P. Gallo, J. Chem. Phys. **144**, 074503 (2016).
[54]O. Mishima and H. E. Stanley, Nature **392**, 164 (1998).
[55]F. Mallamace, C. Corsaro, D. Mallamace, S. Vasi, C. Vasi, H. E. Stanley, and S.-H. Chen, J. Chem. Phys. **142**, 215103 (2015).
[56]R. Richert and A. Blumen, *Disorder Effects on Relaxational Processes: Glasses, Polymers, Proteins* (Springer, Berlin, Heidelberg, 2012).
[57]K. T. Gillen, D. C. Douglass, and M. J. R. Hoch, J. Chem. Phys. **57**, 5117 (1972).
[58]A. Dehaoui, B. Issenmann, and F. Caupin, Proc. Natl. Acad. Sci. U. S. A. **112**, 12020 (2015).
[59]N. H. Fletcher, *The Chemical Physics of Ice*, Cambridge Books Online (Cambridge University Press, 1970).
[60]K. T. Wikfeldt, A. Nilsson, and L. G. M. Pettersson, Phys. Chem. Chem. Phys. **13**, 19918 (2011).
[61]A. Nilsson and L. G. M. Pettersson, Nat. Commun. **6**, 8998 (2015).
[62]Q. Wan, L. Spanu, G. A. Galli, and F. Gygi, J. Chem. Theory Comput. **9**, 4124 (2013).
[63]D. Corradini, P. Gallo, and M. Rovere, J. Chem. Phys. **128**, 244508 (2008).
[64]D. Corradini, M. Rovere, and P. Gallo, J. Chem. Phys. **132**, 134508 (2010).
[65]P. Gallo, D. Corradini, and M. Rovere, J. Chem. Phys. **139**, 204503 (2013).
[66]F. Mallamace, C. Branca, C. Corsaro, N. Leone, J. Spooren, H. E. Stanley, and S.-H. Chen, J. Phys. Chem. B **114**, 1870 (2010).
[67]F. Mallamace, C. Corsaro, P. Baglioni, E. Fratini, and S.-H. Chen, J. Phys.: Condens. Matter **24**, 064103 (2012).
[68]P. G. Debenedetti, *Metastable Liquids: Concepts and Principles* (Princeton University Press, Princeton, USA, 1996).
[69]P. Kumar, K. T. Wikfeldt, D. Schlesinger, L. G. M. Pettersson, and H. E. Stanley, Sci. Rep. **3**, 1980 (2013).
[70]P. Gallo, Phys. Chem. Chem. Phys. **2**, 1607 (2000).
[71]F. Venturini, P. Gallo, M. A. Ricci, A. R. Bizzarri, and S. Cannistraro, Philos. Mag., Part B **82**, 507 (2002).
[72]A. R. Bizzarri, A. Paciaroni, and S. Cannistraro, Phys. Rev. E **62**, 3991 (2000).
[73]Z. Wang, K.-H. Liu, P. Le, M. Li, W.-S. Chiang, J. B. Leão, J. R. D. Copley, M. Tyagi, A. Podlesnyak, A. I. Kolesnikov, C.-Y. Mou, and S.-H. Chen, Phys. Rev. Lett. **112**, 237802 (2014).
[74]A. Cupane, M. Fomina, and G. Schirò, J. Chem. Phys. **141**, 18C510 (2014).
[75]Z. Wang, A. I. Kolesnikov, K. Ito, A. Podlesnyak, and S.-H. Chen, Phys. Rev. Lett. **115**, 235701 (2015).
[76]F. Mallamace, C. Corsaro, D. Mallamace, Z. Wang, and S.-H. Chen, Front. Phys. **10**, 106103 (2015).
[77]F. Mallamace, S.-H. Chen, M. Broccio, C. Corsaro, V. Crupi, D. Majolino, V. Venuti, P. Baglioni, E. Fratini, C. Vannucci, and H. E. Stanley, J. Chem. Phys. **127**, 045104 (2007).
[78]P. W. Fenimore, H. Frauenfelder, B. H. McMahon, and R. D. Young, Proc. Natl. Acad. Sci. U. S. A. **101**, 14408 (2004).
[79]F. Parak and E. W. Knapp, Proc. Natl. Acad. Sci. U. S. A. **81**, 7088 (1984).

[80]S. Combet and J.-M. Zanotti, Phys. Chem. Chem. Phys. **14**, 4927 (2012).
[81]C. Calero, H. Stanley, and G. Franzese, Materials (Basel). **9**, 319 (2016).
[82]A. D. Mackerell, D. Bashford, M. Bellott, R. L. Dunbrack, J. D. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F. T. Lau, C. Mattos, S. Michnick, T. Ngo, D. T. Nguyen, B. Prodhom, W. E. Reiher, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiórkiewicz-Kuczera, D. Yin, and M. Karplus, J. Phys. Chem. B **102**, 3586 (1998).
[83]A. D. Mackerell, M. Feig, and C. L. Brooks, J. Comput. Chem. **25**, 1400 (2004).
[84]H. J. C. Berendsen, J. R. Grigera, and T. P. Straatsma, J. Phys. Chem. **91**, 6269 (1987).
[85]B. Hess, C. Kutzner, D. van der Spoel, and E. Lindahl, J. Chem. Theory Comput. **4**, 435 (2008).
[86]H. J. C. Berendsen, J. P. M. Postma, W. F. van Gunsteren, A. DiNola, and J. R. Haak, J. Chem. Phys. **81**, 3684 (1984).
[87]G. Franzese and V. Bianco, Food Biophys. **8**, 153 (2013).