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SLOW DYNAMICS OF SUPERCOOLED TREHALOSE HYDRATION WATER IN COMPARISON WITH BULK WATER

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ABSTRACT. Trehalose aqueous solutions are relevant in many technological applications, for example in cryopreservation of biomolecules. It is known that the presence of this disaccharide is able to slow down the dynamics of nearby water molecules and to modify their spatial rearrangement, nevertheless, a complete understanding of the properties of water-trehalose solutions and of trehalose cryoprotective properties is still lacking. Here we discuss recent molecular dynamics simulation results of water-trehalose solutions, performed at different temperatures upon cooling, and we compare the results with the behavior of the bulk phase. In particular we focus on the dynamical properties of hydration water, *i.e.*, the water molecules in the hydration shell of the disaccharide. Hydration water shows a sub-diffusive behavior with respect to bulk water, the same structural relaxation typical of glass formers liquids, albeit slightly slower than in the bulk, and an additional relaxation process at longer timescales completely absent in the bulk. The analysis of hydrogen bond autocorrelation functions allows to connect the structural and long relaxation processes of hydration water to the dynamics of two distinct population of hydrogen bonds in the system, the water-water and water-trehalose hydrogen bonds.

1. Introduction

Water is known to have many peculiar behaviors upon cooling (Gallo *et al.* 2016; Gallo and Stanley 2017; Gallo and Sciortino 2019). Depending on the concentration, aqueous solutions have shown to retain some of these behaviors (Corradini *et al.* 2010, 2011; Woutersen *et al.* 2018). Supercooled sugar aqueous solutions are relevant in many technological applications, such as cryopreservation of biological molecules. In particular, trehalose, a disaccharide formed from two glucose rings linked by a 1-1 α-bond, has been proven to be especially effective as a cryoprotectant (Crowe *et al.* 1996; Jain and Roy 2009; Cordone *et al.* 2015). Many different scenarios have been proposed to explain the bioprotective mechanism of carbohydrates, nevertheless, a complete microscopic explanation of the sugars cryoprotecting mechanism is still lacking (Crowe *et al.* 1983; Green and Angell 1989; Belton and Gil 1994; Fedorov *et al.* 2011).

In order to clarify the role of trehalose in biological solutions, extensive experimental and simulations studies have been performed (see for example Magazù *et al.* 1997, 1998;

Branca *et al.* 1999, 2001; Magazù *et al.* 2001; Lee *et al.* 2005; Lerbret *et al.* 2005; Malsam and Aksan 2009; Paolantoni *et al.* 2009; Lerbret *et al.* 2011; Magno and Gallo 2011; Lupi *et al.* 2012a; Lupi *et al.* 2012b; Liu *et al.* 2018; Iorio *et al.* 2019a,b; Iorio *et al.* 2019c). The disaccharide has been shown to alter both structural and dynamical properties of water by destroying its hydrogen-bonds network and by greatly slowing down water relaxation processes upon cooling. While it is well known that bulk water in the supercooled temperature region shows a glassy-like behavior (Gallo *et al.* 1996; Sciortino *et al.* 1996), it is interesting to investigate the impact that the presence of trehalose molecules has on the glassy behavior of surrounding water molecules.

Here we discuss results from Molecular Dynamics simulations of water trehalose solutions at 40.65 wt% concentration of trehalose in the mildly supercooled temperatures range. The focus of the paper is the characterization of the dynamical properties of the so-called hydration water, *i.e.*, the population of water molecules within the hydration shell of the disaccharide (Iorio *et al.* 2019a,b), and the close comparison with the bulk phase behavior. We will also discuss the connection between the translational dynamics of hydration water and the hydrogen bond interaction between water-water and water-trehalose molecules.

2. Methods

We analyze results from classical Molecular Dynamics computer simulations of bulk water and binary mixtures of water and trehalose. The bulk water system is composed by 500 water molecules. The binary mixture is a water-trehalose solution with a concentration of 40.65 wt% in trehalose, composed by 1498 water molecules and 54 trehalose molecules. The SPC/E three sites potential introduced by Berendsen *et al.* (1987) has been used to model the interactions between water molecules, and the CHARMM (Chemistry at HARvard Macromolecular Mechanics) potential has been used for the interactions of the disaccharide molecules (Guvench *et al.* 2008, 2009). Both bulk water and water-trehalose solutions have been studied along the $p = 1.013$ bar isobar for temperatures ranging from 320 K to 200 K. The Berendsen method was used to control both the pressure and the temperature (Berendsen *et al.* 1984) and the GROMACS 4.5.5 package (Hess *et al.* 2008) to run the simulations and to collect the trajectories. For further details on the simulations protocol see Iorio *et al.* (2019a,b).

In this paper we focus on the several correlation functions used to characterize the dynamical properties of water molecules in the bulk and in the hydration shell of water-trehalose solutions: the Mean Square Displacement (MSD), the Self Intermediate Scattering Function (SISF) and the Hydrogen Bonds (HB) autocorrelation function.

The MSD is defined as

$$\left\langle \Delta r^2(t) \right\rangle = \frac{1}{N} \sum_{i=1}^{N} [r_i(t) - r_i(0)]^2 \tag{1}$$

where $r_i(t)$ is the position of the i-th particle at time t and the sum is over the N particles of the system. The MSD is useful to study the amplitude of the particle's motion as a function of the time and it gives a direct way to characterize the diffusive regime of the system under investigation. The long timescales behavior of the MSD follows a power law

$$\left\langle \Delta r^2(t) \right\rangle \sim t^{\alpha}$$

with a Brownian, diffusive regime if $\alpha = 1$, and a sub-diffusive regime for smaller values of the exponent, $0 < \alpha < 1$ (Bouchaud and Georges 1990; Gallo and Rovere 2003).

The SISF probes the single-particle translational dynamics and it is used to characterize structural relaxation phenomena. It is defined as the autocorrelation function of the Fourier components of the local density in the q space

$$F_S(\vec{q},t) = \frac{1}{N} < \rho_{\vec{q}}(t)\rho_{-\vec{q}}(0) >= \frac{1}{N}\left\langle \sum_{i=1}^{N} e^{i\vec{q}\cdot(\vec{r}_i(t)-\vec{r}_i(0))} \right\rangle \tag{2}$$

where $\vec{q}$ is the transferred wavevector, $\vec{r}_i(t)$ is the position of the i-th particle and N is the number of particles in the system. All the SISFs reported in this work are calculated at transferred wavevector $q = |\vec{q}| = 2.25$ Å^{-1}, corresponding to the position of first peak of the oxygen-oxygen structure factor of water, where the features of glassy dynamics of supercooled water are best evident. This value of the wavevector corresponds in fact to the length scale of the cage formed by the first shell of nearest neighbours molecules.

The HB autocorrelation function allows to study the relaxation of the hydrogen bonds network over time. It is defined as (Luzar and Chandler 1996):

$$C(t) = \frac{\langle h(0)h(t)\rangle}{\langle h\rangle} \tag{3}$$

where h is the existence operator of an hydrogen bond. $h(t)$ is equal to 1 if a tagged pair of molecules is hydrogen bonded at time t and it is equal to 0 otherwise. We used a geometric definition for the formation of an hydrogen bond: two molecules are hydrogen bonded if

$$d < 0.35 \text{ nm}$$
$$\theta \leq 30^\circ$$

where d is the donor-acceptor distance and θ is the donor-hydrogen-acceptor angle.

We consider a water molecule to be part of the hydration shell of trehalose if the distance of its oxygen atom from the closest atom of the disaccharide is less than 0.6 nm. According to this definition, the hydration water of trehalose has been shown (Iorio *et al.* 2019b) to consist of roughly two layers of water molecules surrounding the disaccharide.

3. Results and discussion

Figure 1 shows a snapshot of the water-trehalose solution with a concentration of 40.65 wt% of trehalose at $T = 200$ K. At this temperature trehalose molecules appear aggregated to form a cluster with a large surface exposed to water (Iorio *et al.* 2019a). This is in agreement with experimental results showing that the solubility of the sugar approaches zero below $T = 280$ K and at 1 bar (Mehl 1997; Iorio *et al.* 2019a).

At low temperature the clusters observed are composed by more than 80% of the total number of trehalose molecules in the system. This concentration of trehalose molecules in solution has been selected for a close comparison with the bulk phase because in this high limit of concentrations the modifications induced in the dynamics of hydration water are more enhanced (Iorio *et al.* 2019a).

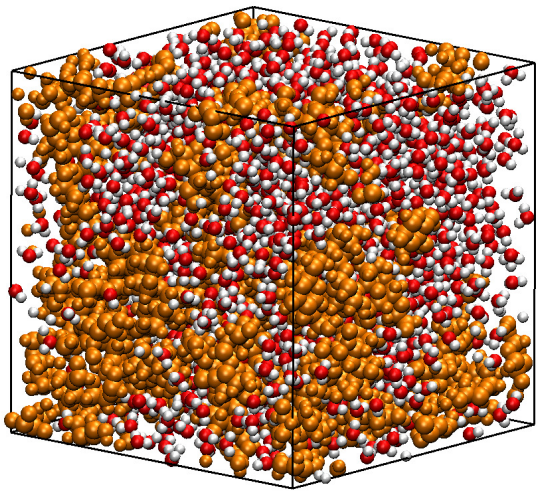

FIGURE 1. Snapshot of the water-trehalose solution at $T = 200\,\mathrm{K}$. Trehalose molecules are depicted in orange, water molecules in red and white.

To investigate the dynamics of hydration water we show in Fig. 2 the Mean Square Displacement (MSD) of the oxygen atoms of the water molecules in the hydration layer of trehalose in comparison with the bulk phase at three selected temperatures, 300 K, 240 K and 200 K. In order to provide information about hydration water, the dynamical quantities are calculated only for the time interval in which a water molecule (in particular its oxygen atom) resides in the 0.6 nm shell around trehalose molecules. The MSD data show an initial ballistic regime for both hydration and bulk water, followed by a plateau region, starting at around 0.3 ps, due to the rattling of the water molecules trapped inside the cages formed by the nearest neighbours upon cooling. In the case of bulk water, at the long timescales the molecules recover the Brownian diffusion. The direct comparison between hydration and bulk water (Fig. 2) shows a marked sub-diffusive behavior of hydration water with respect to the bulk, especially at the two lowest temperatures. The sub-diffusive behaviour has been observed also in many confined systems (Gallo *et al.* 2002; Gallo and Rovere 2003; Gallo *et al.* 2010) and in aqueous solutions of biomolecules (Rocchi *et al.* 1998; Bizzarri 2002; Camisasca *et al.* 2018; Tan *et al.* 2018). In this case sub-diffusivity is due to the interactions of water molecules with trehalose through hydrogen bonds or to steric hindrance that prevents the water molecules from moving freely and to recover the Brownian regime, at least in the time scale of the simulations analyzed.

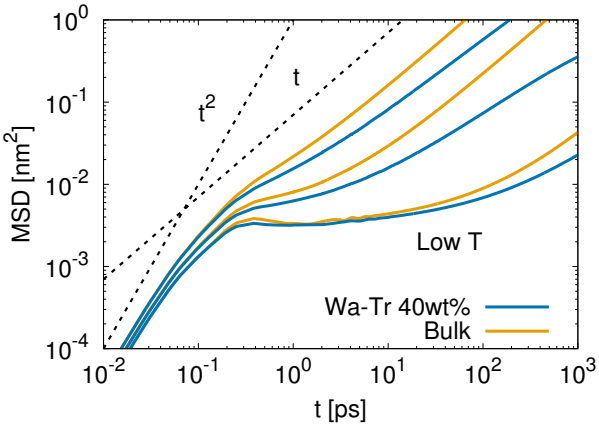

FIGURE 2. Mean Square Displacement for the oxygen atoms of hydration water (blue curves) and bulk water (yellow curves) at three selected temperatures: 300 K, 240 K and 200 K. Dashed black lines are guides for the eyes for the short time ballistic regime (t^2) and diffusive regime (t).

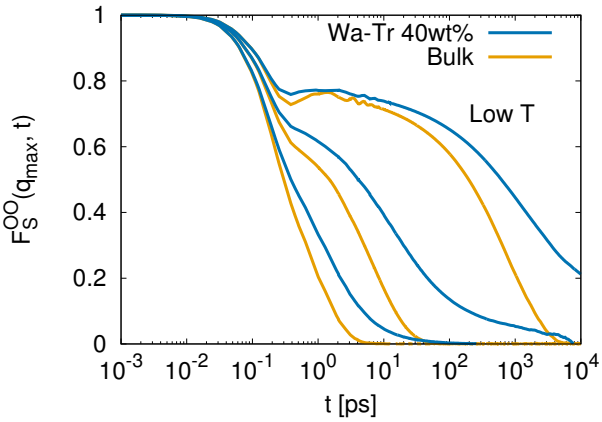

FIGURE 3. Oxygen-Oxygen Self Intermediate Scattering Functions for hydration water (blue curves) and bulk water (yellow curves) at three selected temperatures: 300 K, 240 K and 200 K. Correlators are calculated at $q_{\max} = 2.25$ Å^{-1}.

The translational dynamics of hydration water upon cooling can be further characterized by analyzing the oxygen SISF calculated according to Eq. (2). Figure 3 shows the oxygen-oxygen SISFs of hydration water for the water trehalose solutions at three temperatures, 300 K, 240 K and 200 K, in comparison with the oxygen-oxygen SISFs of bulk water at the same temperatures. The two steps relaxation typical of glass former liquids (Gallo *et al.* 1996; Sciortino *et al.* 1996) is evident for both bulk and hydration water. The initial ballistic motion corresponding to the short-time Gaussian relaxation shows only small changes upon

cooling (Fig. 3). At about 0.3 ps one can observe the plateau region already found in the MSD data, which is more pronounced at the lowest temperatures, followed by the second relaxation, typically corresponding to the relaxation of the cage (α-decay) for glass formers. The α-decay present in both bulk and hydration water shows a characteristic relaxation time strongly dependent on the temperature of the solution. This can be gleaned from Fig. 3 with the correlators stretching to longer timescales as the temperature goes down. The presence of a structural relaxation on a thin layer of water molecules, such as the hydration layer, shows the ubiquitous nature of the glassy behaviour of water in the mild supercooled region. The one-to-one comparison at each temperature between the SISF of bulk water and of hydration water (Fig. 3) also highlights the presence of a long timescale component in the correlators of hydration water, completely absent in bulk water. This long relaxation is the most pronounced with decreasing temperature. Note that the SISF of the hydration water at 200 K does not decay to zero in the timescale of the tens of nanoseconds investigated. The long timescale tail, also found for the case of water-protein solutions (Bizzarri 2002; Comez *et al.* 2013; Corradini *et al.* 2013; Camisasca *et al.* 2016), can be attributed to water-solute interactions. The temperature dependence of the relaxation times of the α and long relaxation processes in hydration water will be discussed later on.

To examine more in depth the origin of the α and long relaxation processes of hydration water we show also the water-water and the water-trehalose hydrogen bond dynamics by analyzing the HB autocorrelation functions $C(t)$, calculated according to Eq. (3). Figure 4 shows the HB correlation functions at the three selected temperatures: 300 K, 240 K and 200 K. Top panel displays the water-water HB correlation functions in bulk water and in the hydration water of trehalose-water solutions. The results show a slighlty slower HB dynamics in the hydration water with respect to bulk water, reasonably due to the overall slightly slower translational dynamics of hydration water with respect to bulk water as obtained from the SISFs correlators. The water-trehalose HB correlations functions (bottom panel of Fig. 4) show a quite slower decay with respect to the water-water HB correlation functions of hydration water, implying that the interaction of water molecules with trehalose is more persistent in time than water-water interactions.

Characteristic timescales of the relaxation processes discussed can be extracted from both SISFs and $C(t)$ functions by fitting procedure of the data. The α- and the long relaxation times can be extracted from the SISF functions by modelling the correlators according to a functional form of the type (Magno and Gallo 2011):

$$F_S(q_{\max},t) = [1 - f_\alpha - f_{\text{long}}]e^{-\left(\frac{t}{\tau_{\text{short}}}\right)^2} + f_\alpha e^{-\left(\frac{t}{\tau_\alpha}\right)^{\beta\alpha}} + f_{\text{long}} e^{-\left(\frac{t}{\tau_{\text{long}}}\right)^{\beta_{\text{long}}}}$$

where the first two terms account for the Gaussian and α structural relaxation processes also present in bulk water (Gallo *et al.* 1996; Sciortino *et al.* 1996) and the last stretched exponential is used to model the long relaxation process observed for water at the interface with macromolecules (Magno and Gallo 2011; Corradini *et al.* 2013; Camisasca *et al.* 2016; Camisasca *et al.* 2018; Iorio *et al.* 2019a; Iorio *et al.* 2019c). Hydrogen-bond relaxation times can be obtained from the HB correlators by fitting the data to a stretched exponential of the form:

$$C(t) \propto e^{-\left(\frac{t}{\tau}\right)^\beta},$$

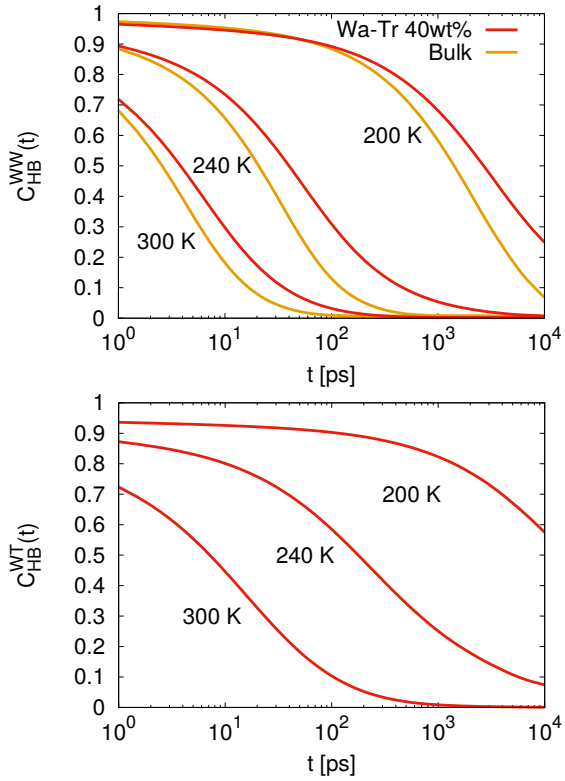

FIGURE 4. Hydrogen bonds correlation functions at three selected temperatures: 300 K, 240 K and 200 K. Top panel refers to water-water hydrogen bonds correlation functions, $C_{\mathrm{HB}}^{\mathrm{WW}}(t)$, of bulk water (yellow curves) and of hydration water (red curves). Bottom panel refers to water-trehalose hydrogen bonds correlation functions, $C_{\mathrm{HB}}^{\mathrm{WT}}(t)$.

where τ is the characteristic relaxation time of the process. The relaxation times τ obtained are presented in the Arrhenius plots of Fig. 5. The top panel shows a comparison between the structural relaxation time τ_α and the water-water hydrogen bond relaxation time $\tau_{\mathrm{HB}}^{\mathrm{WW}}$ for hydration water. The vertical dashed line at $T = 230\,\mathrm{K}$ indicates that the position of the crossover between a fragile and a strong region observed in the dynamics of the hydration water (Iorio *et al.* 2019a,b) is the same for the structural and the hydrogen bond relaxation. This indicates that the HB dynamics is related to the translational dynamics of water molecules. Moreover, the results show a longer relaxation time of the hydrogen bond dynamics with respect to the structural relaxation of the cage, implying a higher persistence in time of the hydrogen bond network.

The lower panel of Fig. 5 shows a comparison between the relaxation times of the water-trehalose HB correlation function and the characteristic time of the long timescale relaxation process observed in the SISFs of hydration water. It can be observed that both τ_{long} and $\tau_{\mathrm{HB}}^{\mathrm{WT}}$ present two well distinct linear trends in Arrhenius plot, with a strong-to-strong crossover.

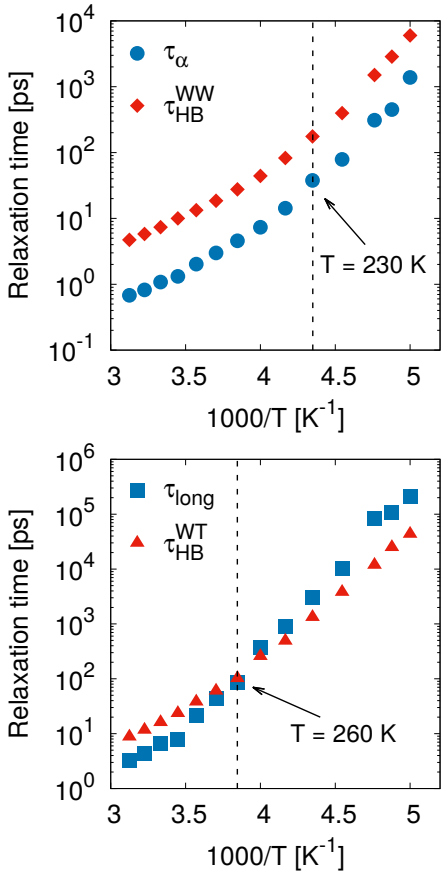

FIGURE 5. Relaxation times extracted from the SISFs and from the hydrogen bonds correlation functions. Top panel reports the structural α-relaxation time of hydration water, τ_α, and the water-water hydrogen bonds relaxation time of hydration water, τ_{HB}^{WW}. Vertical dashed black line at $T = 230$ K represents the position of the crossover from a fragile region to a strong region. Bottom panel reports the long relaxation time of hydration water, τ_{long}, and the water-trehalose hydrogen bonds relaxation time of hydration water, τ_{HB}^{WT}. Vertical dashed black line at $T = 260$ K represents the position of the strong-to-strong crossover

This crossover observed in the long relaxation processes of water-macromolecules solutions has been associated to a dynamical transition similar to the Protein Dynamical Transitions (PDT) also observed for this system (Iorio *et al.* 2019a). The PDT transition has also been observed in neutron scattering experiments (Schirò *et al.* 2015), as well as in simulation works (Kumar *et al.* 2006; Wood *et al.* 2008; Schirò *et al.* 2015; Camisasca *et al.* 2016; Zanatta *et al.* 2018; Tavagnacco *et al.* 2019) not only in proteins, but in a variety of biological and non-biological macromolecules. For all these different systems, PDT occurs between 200 K and 260 K. In Fig. 5 we show that the change of slope occurs at about $T = 260$ K for

both long and water-trehalose HB relaxation processes, showing a clear connection between the dynamical transition and the relaxation of the water-trehalose hydrogen bond dynamics.

The similarity of the temperature dependence between τ_α and τ_{HB}^{WW} and between τ_{long} and τ_{HB}^{WT} allows us to disentangle the two relaxation processes observed in the SISFs correlators of hydration water by associating them to the dynamics of two distinct "populations" of hydrogen bonds, namely the water-water and the water-trehalose hydrogen bonds.

4. Conclusions

We have shown the dynamical properties of water trehalose solutions as a function of temperature, down to the mild supercooled region, in close comparison with the results for bulk water.

Analysis of the Mean Square Displacements of the oxygen atoms of the water molecules in the hydration shell of trehalose shows a subdiffusive behavior with respect to bulk water especially at the two lower temperatures (Gallo *et al.* 2002; Camisasca *et al.* 2018; Iorio *et al.* 2019b). This can be associated to the interactions of water molecules with the disaccharide through hydrogen bonds and to the steric hindrance that prevents water molecules to rearrange freely and to recover the Brownian dynamics.

Self Intermediate Scattering Functions show the existence of two slow relaxations in hydration water (Magno and Gallo 2011; Corradini *et al.* 2013; Camisasca *et al.* 2016; Camisasca *et al.* 2018; Iorio *et al.* 2019a,b; Iorio *et al.* 2019c). The first one corresponds to the structural relaxation also present in bulk water, albeit with a slower relaxation time, and corresponding to the relaxation of the cage for glass formers (α-relaxation). The presence of this structural relaxation in a thin layer of water molecules highlights the ubiquitous nature of the glassy dynamics of water in this temperature region. The second long relaxation, not present in bulk water, is the most pronounced at the lowest temperatures. This long timescales relaxation has also been observed in the case of water-protein solutions and has been ascribed to the water-solute interactions. Moreover, the temperature dependence of this long timescale relaxation process shows a strong-to-strong transition that has been associated to a dynamical transition similar to the well characterized Protein Dynamical Transition (Kumar *et al.* 2006; Wood *et al.* 2008; Schirò *et al.* 2015; Camisasca *et al.* 2016; Zanatta *et al.* 2018; Iorio *et al.* 2019a; Tavagnacco *et al.* 2019).

Hydrogen bonds autocorrelation functions reveal very important information on the two relaxation processes observed from the SISFs. The structural relaxation times of hydration water show a temperature dependence very similar to the one of the water-water hydrogen bond relaxation times in the hydration layer of trehalose. This indicates that the hydrogen bond dynamics is connected to the translational dynamics of the system. Moreover, the relaxation times of water-trehalose hydrogen bond shows a strong-to-strong crossover at the temperature of the long relaxation process, showing a clear connection between the dynamical transition and the water-trehalose hydrogen bond dynamics.

These results strongly confirm the disentanglement of the two relaxations found in the SISFs of hydration water of trehalose solutions and allows to associate them to the two distinct populations of hydrogen bonds in the system; namely the water-water and the water-trehalose hydrogen bonds.

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