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ARTICLE *in* JOURNAL OF PHYSICAL CHEMISTRY LETTERS · MARCH 2014

Impact Factor: 7.46 · DOI: 10.1021/jz500023e

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On the Coupling between the Collective Dynamics of Proteins and Their Hydration Water

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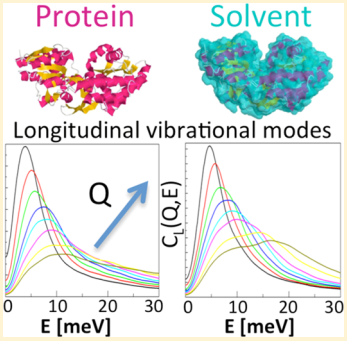
Supporting Information

ABSTRACT: Picosecond time scale dynamics of hydrated proteins has been connected with the onset of biological activity as it coincides with solvent−solute hydrogen bond rearrangements and amino acid rotational relaxation time scales. The presence and fluctuations of protein hydration water (PHW) largely influence protein motions that are believed to be slaved to those of the solvent, yet to date, how protein and hydration water dynamics are coupled remains unclear. Here, we provide a significant advance in characterizing this coupling; we present the first full study of both the longitudinal and transverse coherent collective motions in a protein−solvent system. The data show unexpectedly the presence in the water dynamics of collective modes belonging to the protein. The properties of these modes, in particular, their propagation velocities and amplitudes, indicate a strengthening of the interactions and a higher rigidity of the network of solvent molecules close to the protein surface. Accordingly, the present study presents the most compelling and clear evidence of a very strong dynamical coupling between a protein and its hydration water, previously suggested by studies using various experimental techniques.



SECTION: Biophysical Chemistry and Biomolecules

Water is the native protein environment and the solvent for biological processes.[1,2] A threshold level of hydration of ≃0.2 g of H_2O per g of dry protein is believed to be a necessary condition for protein function.[3] Furthermore, water that solvates proteins, that is, that resides within a few Å of their surface, referred to as protein hydration water (PHW), has been shown to play a role in efficient enzymatic catalysis,[4] in folding processes,[5−7] as well as in molecular recognition and in mediating protein−protein interactions.

The dynamics of PHW has been studied by means of several experimental and theoretical techniques such as X-ray and inelastic neutron scattering,[8−11] depolarized light scattering,[12,13] terahertz spectroscopy,[14,15] femtosecond fluorescence,[16] and molecular dynamics (MD) simulations,[11,17−20] to name a few. Within the wide range of time scales in which the coupling between PHW and the proteins extends,[21] special attention has been paid to the picosecond processes that seem to be connected with the onset of biological activity[22] as hydrogen bond rearrangements and rotational relaxation occur on this time scale.[4,23] Previous investigations[23−26] have shown that the fluctuations of PHW largely influence certain protein motions that are therefore "slaved" and tightly coupled to the solvent. Conversely, structural features and single-particle dynamics of PHW are significantly perturbed with respect to the bulk as they are affected by the local topography and specific interactions with the protein,[8,28−30] resulting in solute-induced retardation of the PHW rotational and diffusive motions in proximity to proteins.[18,24]

Relatively few studies have probed PHW collective dynamics.[9,18,31−33] The majority have examined the solvent longitudinal modes by investigating the properties of the dynamical coherent structure factor $S(Q,E)$ that can be probed by inelastic neutron or X-ray scattering. In contrast to what has been highlighted for single-particle dynamics, it was reported that PHW collective dynamics bears close similarity to that of liquid water, that is, the presence of the protein has negligible effects on the longitudinal modes of the solvent.[9,18,31] Very recently, a MD simulations study of a protein solution has provided evidence for long-ranged collective protein−water vibrations at far-infrared/THz frequencies. By analyzing correlated vibrational motions between atoms of the protein surface and hydration water, Heyden and Tobias[32] revealed a dynamical coupling not identified in previous Brillouin neutron scattering (BNS)[9] and MD[18] studies. Details of the dynamical coupling, such as the origin of the correlated vibrations, as well

Received: January 5, 2014
Accepted: March 18, 2014
Published: March 18, 2014

   dx.doi.org/10.1021/jz500023e | J. Phys. Chem. Lett. 2014, 5, 1181−1186

as the spatial dependence of collective density fluctuations in the PHW, remain to be elucidated.[32]

This Letter builds on previous investigations by thoroughly characterizing the coupling between the dynamics of a protein and its hydration water. We report a MD simulation study of a hydrated protein crystal in which we have separately investigated the coherent collective dynamics of the protein and its PHW. In particular, we have investigated the properties of the longitudinal and transverse collective modes propagating in the system by characterizing the correlation functions $C_\alpha(Q,t)$ of the longitudinal (α = L) and the transverse (α = T) currents determined at a wave vector $\mathbf{Q}$. $C_L(Q,t)$ and $C_T(Q,t)$ are defined respectively as $C_\alpha(Q,t) = \langle \mathbf{J}_\alpha^*(\mathbf{Q},t)\cdot\mathbf{J}_\alpha(\mathbf{Q},t)\rangle$, where $\mathbf{J}_L(\mathbf{Q},t) = N^{-1/2}\sum_i \hat{\mathbf{Q}}(\hat{\mathbf{Q}}\cdot\mathbf{v}_i(t))\exp(-i\mathbf{Q}\cdot\mathbf{r}_i(t))$ and $\mathbf{J}_T(\mathbf{Q},t) = N^{-1/2}\sum_i \hat{\mathbf{Q}}\times\mathbf{v}_i(t)\exp(-i\mathbf{Q}\cdot\mathbf{r}_i(t))$, $\hat{\mathbf{Q}}$ being the unit vector along $\mathbf{Q}$; $\mathbf{r}_i(t)$ and $\mathbf{v}_i(t)$ are the positions and velocities of atom i, respectively, and N is the total number of atoms.[34] $C_L(Q,E)$, the time Fourier transform of $C_L(Q,t)$, probes the longitudinal modes, that is, the collective density fluctuations in the system at a given wave vector. $C_L(Q,E)$ is directly related to the dynamical coherent structure factor, $S(Q,E)$, that can be probed by inelastic neutron or X-ray scattering by $C_L(Q,E) = E^2S(Q,E)/Q^2$. In contrast, the transverse current spectra, $C_T(Q,E)$, probes the shear modes propagating in the system at a given wave vector. In general, the presence of collective modes is signaled by the appearance of peaks in the $C_\alpha(Q,E)$ spectra. By reporting the frequencies of these peaks as a function of Q, we evaluate the dispersion curves for the longitudinal and transverse modes, thereby obtaining key information on the nature (acoustic-like or optical-like) of these collective modes.

We considered the maltose binding protein (MBP) in its ligand-free conformation in a crystal-like packing at h = 0.42[10,11] at 150 K (see the Supporting Information (SI) for details). This hydration level is close to that often used in inelastic scattering experiments and comparable to the above-mentioned studies.[9,11,18,35] The MD simulations were carried out using NAMD2.[36] The system was first equilibrated at constant temperature and constant pressure (1 atm) using 3d periodic boundary conditions and employing Langevin dynamics for temperature control and a Nosé–Hoover–Langevin piston for pressure control.[37] Long-range, electrostatics forces were computed using the smooth particle mesh Ewald approach.[38] The CHARMM22[39] force field was used for the protein, and the SPC/E[40] model was used for water. Further details about the simulation protocols may be found in the SI and in previous work.[10] Following more than 10 ns of MD simulation at constant pressure, a 150 ps run was performed at constant volume, and the atomic positions and velocities, saved every femtosecond, were collected for the analyses. Here, we have probed water dynamics specifically by singling out only the contribution from water oxygen atoms. We refer to the investigated selections as water-2.5, water-3, and water-4 to designate water molecules whose oxygen atom is located within 2.5, 3, and 4 Å, respectively, of the nearest MBP atom. These water selections correspond to, respectively, 30, 79, and 95% of the solvent present in the system, the class water-tot representing all water molecules in the system.

At the temperature that we set, which is well below the so-called protein dynamical transition, there is no exchange between the different hydration selections as translational diffusion is completely inhibited.[18,27] Collective vibrational properties of PHW and MBP are not expected to change significantly as the temperature increases.[28] Leu et al. have, for instance, recently shown using X-ray scattering that the longitudinal branch of collective motions in hydrated cytochrome C hardly vary in the 150–300 K range.[41] Moreover, both experiments and simulations[20,32] revealed that a dynamical coupling between a protein and its hydration water persists up to room temperature.

The current spectra for the different water selections calculated at Q = 0.2 Å^{-1} are reported in Figure 1b and c.

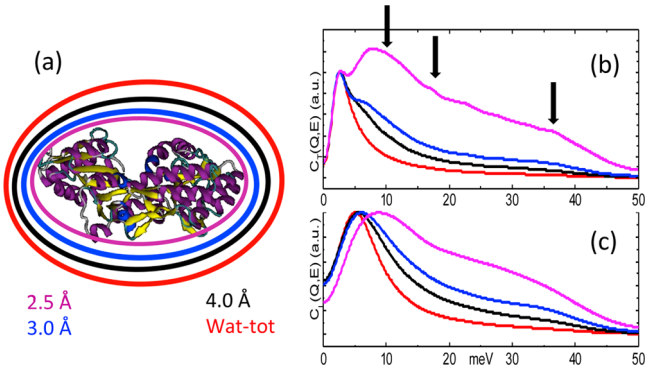


Figure 1. Transverse ($C_T(Q,E)$) (b) and longitudinal ($C_L(Q,E)$) (c) current spectra estimated at a wave vector Q = 0.2 Å^{-1} for maltose binding PHW selections within 2.5, 3.0, and 4.0 Å of the protein and all water molecules (water-tot) in the crystal. The spectra have been normalized to the first peak maximum to facilitate the comparison; the vertical arrows indicate the position of high-energy modes not present for bulk water.

Both $C_L(Q,E)$ and $C_T(Q,E)$ strongly depend on the water molecule's location with respect to the protein interface. The spectra for water-tot resemble those of bulk liquid water,[42] that is, they show one high-intensity mode located at ∼2.5 meV in $C_T(Q,E)$ and one at ∼6 meV in $C_L(Q,E)$, as also found in previous investigations.[9,18] In contrast, the spectra corresponding to the selections of water molecules that are closer to the protein surface (water-4, water-3, and water-2.5) reveal different characteristics; in addition to the low-frequency modes at 2.5 and 5 meV, new spectral features (denoted in Figure 1b by vertical arrows) appear at higher energies, which are increasingly noticeable for molecules closest to the protein. It is worth noting that as the distance of the water molecules from the protein surface decreases, the relative intensities of the lowest-frequency peaks in both in $C_T(Q,E)$ and $C_L(Q,E)$ decrease, whereas the intensities of the higher-energy peaks increase. In particular, for water-2.5, a broad inelastic feature is clearly visible at ≃8.5 meV in both $C_T(Q,E)$ and $C_L(Q,E)$.

To clarify the nature of these modes, which were not identified in previous studies, we focus on the signals from the closest water molecules to the protein (water-2.5 selection). The transverse and longitudinal current spectra estimated at different low Q values and at E < 15 meV are displayed in Figure 2. The $C_T(Q,E)$ spectra clearly show that as Q increases, the position of the lowest-energy peak shifts toward higher energies, disclosing the propagating character of the related mode, while the position of the second peak seems to be centered at about 8.5 meV irrespective of the value of the momentum transfer Q. The Q-dependent behavior of the $C_L(Q,E)$ spectra is similar, even if the trend is less clear as the low-energy peak almost merges with the one at 8.5 meV. The analyses of the current spectra for E > 15 meV (data not

dx.doi.org/10.1021/jz500023e | J. Phys. Chem. Lett. 2014, 5, 1181–1186

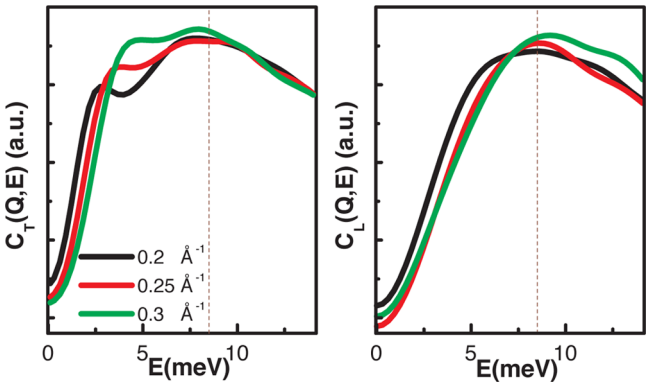


Figure 2. Transverse $C_T(Q,E)$ (left) and longitudinal $C_L(Q,E)$ (right) spectra of the collective modes from water-2.5 at selected Q values. The spectra have been arbitrarily normalized for proper comparison.

shown) have revealed that all other inelastic features above 15 meV have a Q-independent character; they are essentially located at the same energies as they are in the spectra shown in Figure 1b.

The direct analyses of the collective modes of PHW extracted from the simulations reveal the existence of one longitudinal and one transverse propagating mode, as in liquid water,[42] plus optical-like (nonpropagating) features not found in water. These modes are not intense enough when considering the signal from all water molecules in the system and are more prominent in the transverse currents, which probably explains why they were not identified in previous studies that probed longitudinal collective modes of PHW.

We investigated the origin of such optical-like modes by studying the collective motions of the protein itself. The currents describing the modes of several atom types are reported in Figure 3a and b at $Q = 0.2$ Å^{-1}. All $C_T(Q,E)$ and $C_L(Q,E)$ spectra display an intense low-energy peak at energies close to those of the propagating modes in the PHW (cf. Figure 1). At higher energies, other well-defined nonpropagating modes are also visible in the spectra of some species, for example, oxygen and nitrogen. These modes become more visible for carbon atoms as Q increases (Figure 3c). Two features are noticeable; (i) the lowest-energy mode has a dispersive character, whereas all of the modes above 10 meV appear to have an optical-like nature, as seen, for instance, in the $C_L(Q,E)$ for oxygen atoms (Figure 3d), and (ii) most importantly, the energies of the optical-like modes found in the protein spectra show a strict correspondence with those found in water-2.5 (Figure 3c). This is clear evidence that the optical-like modes emerging in the PHW as the protein surface is approached (and not present in pure water) are due to the direct coupling with the vibrational modes of the protein.

With the aim of providing more detailed information on the collective motions in the hydrodynamic regime, that is, at the low dynamical (Q,E) range studied here, we have fitted the $C_L(Q,E)$ and $C_T(Q,E)$ spectra of the carbon atoms of the protein, of water-2.5, and of water-tot for $Q < 0.6$ Å^{-1}. Each of the observed modes (see Figure 3) has been described by a damped harmonic oscillator (DHO) function.[35] Within the framework of hydrodynamics, the DHO function is a simplified version of the generalized three effective eigenmode model.[43,44] It may be expressed as $A(Q)(\Gamma(Q)\Omega^2(Q)E^2)/([E^2 - \Omega^2(Q)]^2 + [\Gamma(Q)E]^2)$, where $\Gamma(Q)$ is the energy width, $\Omega(Q)$ the average excitation energy, and $A(Q)$ the amplitude. Overall, three DHOs with energy below 15 meV were needed to quantitatively reproduce each of the $C_\alpha(Q,E)$ spectra of both protein carbons and PHW at $Q < 0.6$ Å^{-1} (cf. the SI for details). The DHO frequencies obtained from the fitting procedure were subsequently used to determine the corresponding dispersion curves.

Focusing first on the dynamical properties of water-2.5, the data (Figure 4a) indicate that two of the three modes in each of the $C_\alpha(Q,E)$ spectra have an acoustic-like character, that is, they exhibit a steady energy increase with Q, while the third one is associated with an optical branch characterized by an almost constant energy. In analogy to what has been found in liquid water[42] and glassy solids,[45] the dispersion curves derived from the longitudinal and the transverse current spectra superimpose, thus providing a strong indication for the mixed symmetry character of the polarization of the observed branches. Interestingly, we find that the vibrational modes in PHW show a mixed character starting from the lowest

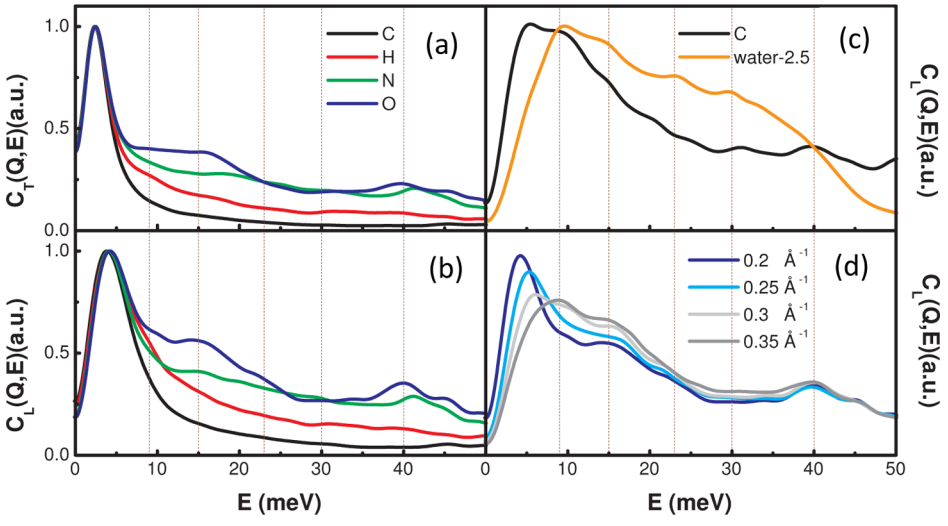


Figure 3. (a) $C_T(Q,E)$ and (b) $C_L(Q,E)$ spectra of selected atom types of MBP calculated at $Q = 0.2$ Å^{-1}. (c) $C_L(Q,E)$ spectra of MBP carbon atoms and of water-2.5 calculated at $Q = 1.35$ Å^{-1}. (d) $C_L(Q,E)$ spectra of carbon atoms of MBP at selected Q values. The vertical dashed lines are indicators for the position of the most prominent optical-like modes.

dx.doi.org/10.1021/jz500023e | J. Phys. Chem. Lett. 2014, 5, 1181−1186

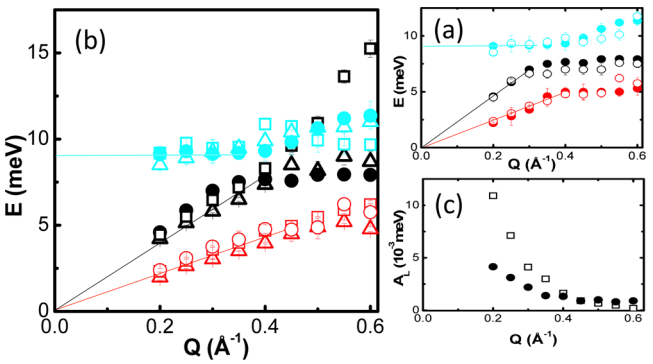


Figure 4. (a) Dispersion curves of water-2.5 derived from $C_T(Q,E)$ (empty symbols) and from $C_L(Q,E)$ (full symbols). (b) Dispersion curves of carbon atoms (triangles), water-2.5 (circles), and water-tot (squares) derived from the transverse spectra $C_T(Q,E)$ (red) for the lowest mode (TA mode) and from the lowest two modes (black and cyan) derived from the longitudinal spectra $C_L(Q,E)$. The lines in (a) and (b) are eye guides. (c) Amplitudes $A(Q)$ of the LA mode derived from $C_L(Q,E)$ for water-2.5 (circles) and water-tot (squares).

investigated Q value (0.2 Å^{-1}), thus indicating a higher degree of disorder of PHW with respect to pure water, in which the pure symmetry character of modes is lost only above Q = 0.6 Å^{-1}.[42]

The high-energy acoustic mode (black symbols in Figure 4a) is the main propagating excitation in the longitudinal current spectra (cf. Figure 4c), though it also appears in $C_T(Q,E)$. Likewise, the low-energy acoustic mode (red symbols in Figure 4a) is the dominant propagating excitation in the transverse current spectra, although it also contributes to $C_L(Q,E)$ as a less intense spectral feature. We therefore refer to these modes, respectively, as the LA and TA modes. In Figure 4b, the dispersion curves of water-2.5 (namely, the dominant propagating modes in $C_T(Q,E)$ and $C_L(Q,E)$: the TA and LA modes together with the optical-like mode) are compared to those of the protein carbon atoms and to those of water-tot. The dispersion curves of the three investigated systems indicate that the collective motions in the whole system are strongly correlated. Most noticeably, the low-energy optical mode is the protein signature found in its hydration water dynamics. This proves that proteins perturb PHW collective dynamics, resulting in vibrational features that differ from those of liquid water, more so as the water−protein distance decreases (see Figures 1 and 4c) but persisting also at larger distances (i.e., in water-tot). It is also important to highlight the fact that many more optical-like modes (detected at higher frequencies) originating from protein collective dynamics are also found in PHW, as can be gathered from Figure 3c. This is perhaps the most significant manifestation that there is indeed a strong coupling between the collective dynamics of a protein and its hydration water. Quite interestingly, the similarity of the acoustic-like modes in the protein and PHW shown here supports the recent study by Heyden and Tobias,[32] which provided evidence for two distinct modes in the protein−water velocity cross-correlation spectra located at the same regime as the HB bending motions in water and the protein−water HB stretch vibrations.

Further examination of the dispersion curves allows one to gain more insight into the protein−water dynamical interplay and to stress differences between the dynamical properties of PHW and those of liquid water. The sound velocities associated with the water TA and LA modes have been derived by evaluating the slopes of the acoustic dispersion curves in the low Q regime. These amount to $\nu_{T-water\text{-}2.5} \simeq 1876 \pm 15$ m/s, $\nu_{T-water\text{-}tot} \simeq 1802 \pm 12$ m/s, $\nu_{L-water\text{-}2.5} \simeq 3537 \pm 45$ m/s, and $\nu_{L-water\text{-}tot} \simeq 3319 \pm 17$ m/s. The data indicate a 7 and 4% increase of ν_L and ν_T, respectively, as the protein surface is approached (cf. Figure S3, SI). The variation of the longitudinal sound mode velocity (ν_L) increases to 16% when we compare $\nu_{L-water\text{-}2.5}$ to the high-frequency sound velocity of 3040 (±80 m/s) in liquid water.[46] This water sound velocity increase as the solvent approaches the protein surface and the (≃15%) increase of the density of the protein first hydration shell compared to bulk water[8,29] both support the picture of a more rigid water network close to the protein surface, which in turn would foster the propagation of collective density fluctuations. Moreover, the vibrational amplitude of the LA mode of water-2.5 is smaller than that of water-tot (see Figure 4c). This is consistent with the fact that the interfacial bound water molecules form stronger hydrogen bonds with the protein amino acid residues.[47] Additionally, in agreement with Heyden et al.,[32] we observe that the vibrational mode at ≃8.5 meV in water-2.5 is blue-shifted in comparison to water-tot (see Figure S2, SI). This shift has also been interpreted as an indication of a strengthening of the interactions in water molecules close to the protein surface.[48]

Specific analyses, such as studying solvent dynamics near large hydrophobic amino acid patches[49] under similar conditions, are needed to determine if the correlations concern only hydrogen-bonded water. However, altogether, the picture that emerges is that hydrogen bonding between PHW and the solute rigidify the protein−water network, allowing for protein collective modes to propagate through the nearby solvent.

In summary, in contrast to former findings[9,18] and due to the full characterization of the longitudinal and transverse modes propagating in a protein crystal-like packing (Figure S1, SI), we have shown that the coherent collective dynamics of PHW significantly differs from that of liquid water. Several previous studies using various approaches including the recent THz spectroscopy technique[48] have shown that PHW is influenced by the protein proximity, and likewise, protein dynamics is influenced by the presence of its PHW. The results presented here provide further and new evidence of a clear coupling between the collective dynamics of the protein and that of its PHW. Real space analyses show that many properties of PHW change with respect to bulk water. It was also often claimed that protein is slaved to water as the latter is requisite to the onset of its dynamical transition. What the present analysis allows us to show is that water does in fact carry protein internal vibrational modes, providing therefore a substantial additional insight about the coupling that would not be available from standard single-particle studies. Furthermore, a quite heterogeneous elastic behavior is shown by the network of water molecules, whose rigidity increases as the protein surface is approached. Although the simulations have been carried out here at 150 K, well below the dynamical transition of the protein to minimize the effect of large motions that can damp the collective modes, and as the origin of the coupling between the protein and its solvent stems mainly from the strong interaction (HB) between the protein and water molecules at its immediate vicinity, one expects these couplings, in light of recent findings,[32] to occur also at room temperature.

dx.doi.org/10.1021/jz500023e | J. Phys. Chem. Lett. 2014, 5, 1181−1186

■ ASSOCIATED CONTENT

Supporting Information

MD simulation methods, details of the analysis procedures, and details of the fitting procedure. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

V.C.N. has carried out research under an HPC-EUROPA2 project. Calculations were performed at the CINES supercomputer center (Montpellier, France). The authors are grateful to Salvatore Patanè for help with data fitting.

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