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Role of the hydrophobic and hydrophilic sites in the dynamic crossover of the protein-hydration water

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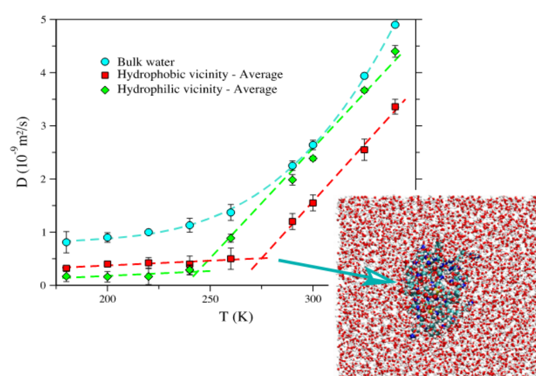
HIGHLIGHTS

- The water diffusion near the protein surface is lower than in bulk.
- A crossover in the diffusion of the hydration water is observed.
- The crossover happens at different temperatures for hydrophilic and hydrophobic sites.

GRAPHICAL ABSTRACT



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ABSTRACT

Molecular dynamics simulations were performed to study the water structure and dynamics in the hydration shell of the globular TS-Kappa protein. The results show that for a wide range of temperatures the diffusion coefficient of water near the protein surface is lower than in bulk. A crossover in the diffusion behavior of hydration water is observed at different temperatures for hydrophilic and hydrophobic vicinities. We have found a correlation between the crossover in the hydrophilic case and the protein dynamical transition. An explanation in terms of the competition between water–water water–protein H-bond formation is provided based on H-bond network analysis.

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1. Introduction

Almost all biomolecules are activated in water environment. In most cases instead of being just an involving layer which increases the solubility, hydration water is an active participant in the dynamics of these molecules [1]. Then, the water first layer and the biomolecule should not be considered as separated entities but form a biologically active entity [2].

One important example is the protein dynamical transition (PDT), recognized as the abrupt increase in the amplitude of the atomic motions observed experimentally in a number of proteins between 180 and 240 K [3–5]. This phenomena depends on the hydration water because the conformation and the stability of the proteins can only be retained in the presence of a critical amount of water, the hydration shell. Consequently, the decrease of the hydration level around the biomolecule leads to the suppression of both the PDT [6,7] and of the biological activity [8,9]. This happens because the water layer and the protein are connected by a hydrogen-bonded (HB) network [10]. Furthermore, the protein dynamical transition, which depends on the relaxation of the protein–water HB network, is affected by the water translational diffusion [11]. Moreover, since the HB network changes with the addition of other molecules in the solution, the PDT shifts to higher temperatures when the solvent viscosity is increased [6,12]. It is clear that water affects the protein through the increase of both the translational and of the rotational dynamics of water [13,14], which is evidenced by the fragile-to-strong crossover in the Arrhenius plot of the water average relaxation times [15–17].

As one entity, the protein also affects the water around it. NMR and neutron scattering experiments, by probing the average atomic motion, shows that the dynamic and the structure of the water in the hydration shell is quite different from the bulk water [18–24]. The simulations indicate that the water in the protein hydration shell exhibits not only a slower mobility but also a heterogeneous dynamics with a sublinear diffusion [25]. This slow dynamics can be correlated with superficial water–water HB that form a network hindering the water diffusion [26]. The disruption of this network enhances the dynamics. The function of the hydrophobic and the hydrophilic sites in this disruption, however, remains not clear.

In this work we address the role of the hydrophobic and of the hydrophilic regions of the protein in the hydration water dynamics and, consequently, in the protein dynamical transition. Molecular dynamics (MD) simulations of the globular TS-Kappa protein [27], immersed in SPC/E [28] water are performed and the mobility of the hydration shell is computed. The TS-Kappa is selected because it has both hydrophobic and hydrophilic sites randomly distributed in the protein surface. This allows us to explore the local dynamics.

The paper is organized as follows. In the Section 2, the computational details and the methods are described, in the Section 3 the main results of the dynamic and structural properties of the water–protein system are discussed. Conclusions are presented in the Section 4.

2. Computational details and methods

Molecular dynamics simulations of solvated TS-Kappa protein using GROMACS [29] 4.5.5 package were performed. We have adopted a globular protein. Even though the surface area accessible to solvents is smaller in globular than in intrinsically disordered proteins [30], the connection between the hydration translational diffusion and the protein dynamical transition does not depend if the protein is intrinsically disordered or folded [11]. This independence between shape and dynamics is also observed in comparing the mobility of water in different globular proteins [31]. This result suggests that in principle it is possible to learn about the dynamics of the proteins by analyzing one specific structure.

In order to describe bonded and non-bonded interactions within the TS-Kappa protein, the OPLS-AA force field [32] was used. Long-range coulomb interaction was handled using the particle mesh Ewald (PME) summation and the non-bonded interactions were truncated at 1 nm.

The system comprises one TS-Kappa protein (1TSK at the Protein Data Bank [33]) solvated by 3950 SPC/E water molecules in a cubic box of side 5.5 nm, as shown in the Fig. 1(a). The protein has thirty-five residues: eight with hydrophobic behavior and twenty-seven hydrophilic mixed. For the definition of hydrophobic and hydrophilic sites we follow the polarity criteria in Ref. [33]. The geometry of water molecules was kept by the SHAKE algorithm [34] and periodic boundary conditions were employed in all directions. After solvated, the output file contains a charged protein, with a net charge of $+5e$. In order to guarantee no net charge in the unit cell, five Cl^- were added.

The simulation run was performed in three steps as follows. Initially the system was equilibrated during 5 ns with the protein atoms kept fixed and water and ions free to move. Then, more 5 ns of simulations were performed, but this time with all atoms free to move. Finally, a 10 ns production run followed. The entire simulation was implemented in the NPT ensemble. For each run, the pressure was kept at 1 atm by the Parrinello–Rahman barostat [35] while temperature was controlled by the Nosé-Hoover thermostat [36,37]. The timestep for the simulations was 2 fs and data were recorded every timestep, for further analysis purposes.

The hydrogen bond was defined by the geometrical criterion of donor-hydrogen-acceptor (DHA) angle and donor–acceptor (DA) or hydrogen-acceptor (HA) distance. Here, we considered a maximum DHA angle of 30° and a DA distance of 0.35 nm, corresponding to the first minimum of the radial distribution function of SPC/E water [38]. The first hydration shell was defined as the water molecules at a distance minor or equal than 0.4 nm [25,39]. For the change of the dynamics of the water due to the presence of the protein only the first hydration shell was taken into account since the perturbation induced by a biomolecule falls off rapidly with distance from the surface [40,41].

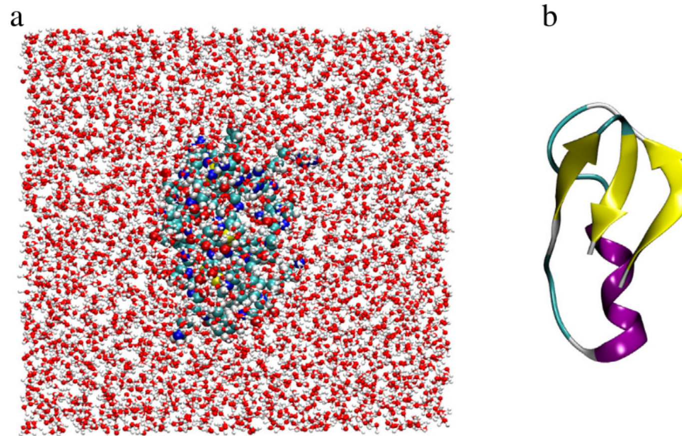

Fig. 1. (a) Snapshot of the simulated system and (b) TS-Kappa protein depicted as a ribbon, colored on the basis of the secondary structure. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

For the lifetime estimation an auto-correlation function based on the distribution of lifetimes $\mathrm{P}(\tau)$ is constructed:

$$C(t) = 1 - \int_0^t \mathrm{P}(\tau)\mathrm{d}\tau. \tag{1}$$

The HB lifetime τ can now be associated with the integral of Eq. (1):

$$\tau = \int C(t)\mathrm{d}t. \tag{2}$$

The self-diffusion coefficient D, averaged over all particles was determined from the Einstein relation [42] as the mean squared displacement (MSD) along the trajectory of a particle

$$D = \lim_{t\to\infty} \frac{\left\langle \left|\vec{r}(t) - \vec{r}(0)\right|^2 \right\rangle}{2dt} \tag{3}$$

where $\langle\rangle$ denotes an average over all the molecules, d stands for dimensionality and $\vec{r}\,(t)$ is the displacement of a molecule during the time interval t. For the diffusion of water at the protein surface, only the molecules that remains for at least 70% of the simulation time in the vicinity of the site (hydrophobic or hydrophilic) are considered for the MSD computation and further diffusion calculations.

3. Results and discussion

First, the protein–water interaction is computed by analyzing the mobility of the water at the first hydration shell close to the hydrophobic and the hydrophilic sites. The Fig. 2 illustrates the variation with temperature of the average diffusion coefficient of the water molecules close to the hydrophobic (squares) and to the hydrophilic (diamonds) patches of the protein. Our results are consistent with the experiments which show that the overall mobility of the hydrated water is lower than the diffusion of the bulk water [43]. Simulations have also shown that the rotational diffusion and relaxation times are lower in the hydration layer of small peptides, proteins and cells compared to the bulk [44]. In this studies, the evolution of the orientational relaxation time, integrated over the rotational auto-correlation function, is proportional to the translational diffusion coefficient [45,46].

The behavior of D with the temperature for the bulk and for the hydration water are quite distinct. While the diffusion coefficient of the bulk water exhibits a parabolic increase with the temperature, the diffusion coefficient of the hydration water shows two linear regimes with different slopes and a crossover between them. For the water in the vicinity of the hydrophobic sites of the protein, the crossover from almost zero mobility to linear diffusion occurs at a temperature of about 270 K. In the case of the hydration water close to the hydrophilic sites the crossover happens at a temperature of about 240 K.

The crossover at 240 K was observed before by analyzing the whole hydration layer [7,14,19] around the protein. Its origin is still under debate. In some cases this transition is associated with the low density liquid to high density liquid crossover observed in confined super-cooled water [15,17,47]. In other cases, no sign of a fragile-to-strong transition is observed and the crossover is interpreted as a kinetic consequence of the glass transition at lower temperatures [48].

Our results illustrated in the Fig. 2 adds complexity to this picture since instead of having one crossover temperature it presents two distinct transitions for the hydrophobic and for the hydrophilic sites. In order to explore the differences

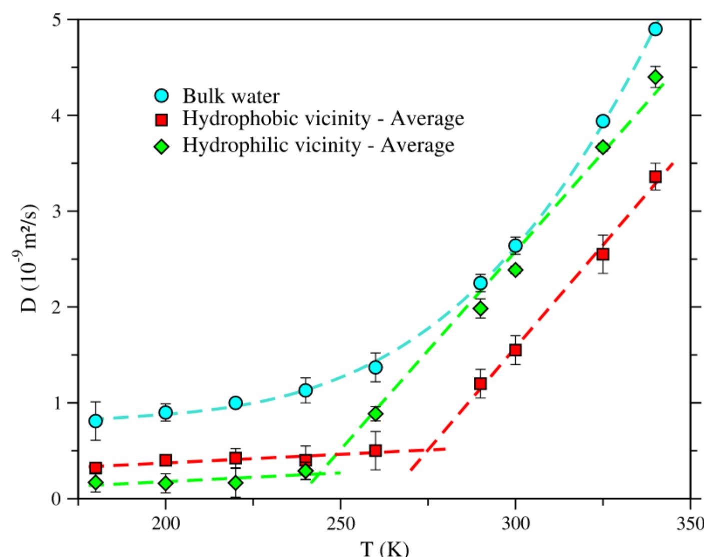

Fig. 2. Diffusion coefficient *versus* temperature for water in bulk (circles) and in the vicinity of hydrophobic (squares) and hydrophilic (diamonds) amino acids. The dashed lines are fit curves between the points.

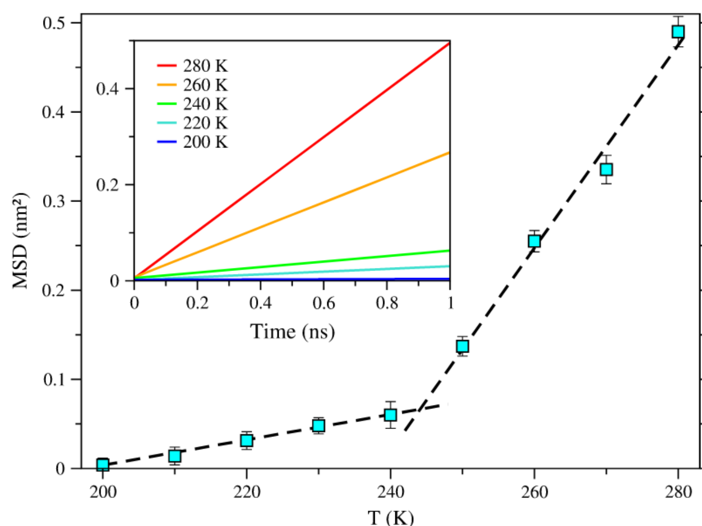

Fig. 3. Mean squared displacement of TS-Kappa's hydrogen atoms *versus* temperature. The dashed lines are fit curves between the points. The inset shows the MSD time evolution for some selected temperatures.

between the two mechanisms the mean square displacement of the TS-Kappa's hydrogen atoms was computed. The hydrogen are the lightest and most mobile atoms over the protein surface, therefore a useful probe of the mobility of the TS-Kappa as a whole. In order to completely remove the translational motions of the protein during the simulation, and access just the fluctuations in its structure, we compute the MSD at each time step by comparing the protein structure at time t with respect to the reference initial configuration of the protein through a least-squares fitting of the two compared structures. The time dependent hydrogen's MSD is then averaged over 1 ns, as shown in Fig. 3.

There are two main contributions to the protein's MSD: a vibrational component predominant at low temperatures and a conformational component activated by temperature and absent in dry proteins [49]. The Fig. 3 illustrates the protein MSD dynamic crossover around $T = 240$ K. This is the same temperature in which the dynamic crossover in the water diffusion at the hydrophilic sites is shown in the Fig. 2. At low temperatures the protein exhibits a very slow dynamics also observed in other studies [7,50]. The transition at 240 K might be due to the coupling between water and protein kinetics [5,11,14,17,48].

In order to investigate if the transition at 240 K could be related to this kinetic coupling effect, the number of HBs (n_{HB}) is evaluated. The Fig. 4 shows the number of water–water ($n_{\mathrm{w\text{-}wHB}}$) and water–protein ($n_{\mathrm{w\text{-}pHB}}$) hydrogen bonds as a function of the temperature. The number of water–water hydrogen bonds is lower than the number of water–protein bonds in the

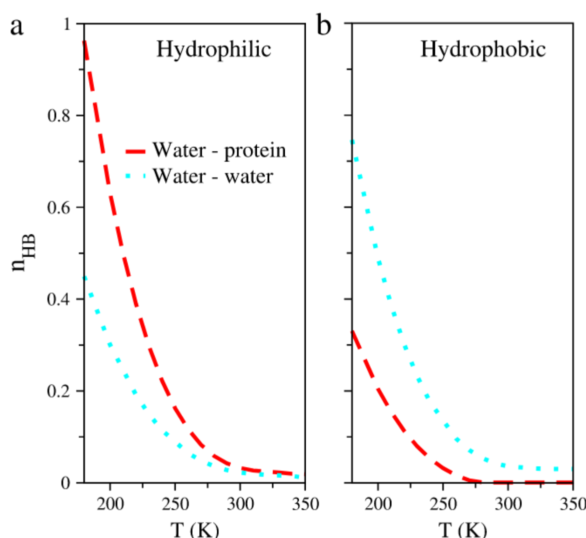

Fig. 4. Number of water–water (dotted line) and water–protein (dashed line) HBs per water molecule *versus* temperature averaged over the vicinity of the (a) hydrophilic and the (b) hydrophobic amino acids.

vicinity of the hydrophilic sites which indicates that the hydrophilic patches of the protein attract water molecules to its surface breaking water–water HBs, as suggested in our previous work [51]. The water–water and water–protein hydrogen bonds show a very similar behavior converging to the same value around 300 K.

In the hydrophobic region, the behavior is the opposite. The number of water–water hydrogen bonds is larger than the number of water–protein bonds, $n_{\text{w-wHB}} > n_{\text{w-pHB}}$, indicating that the water molecules prefer to connect with each other rather than with the protein amino acids. As illustrated in the Fig. 4(b) for temperatures higher than 270 K almost no bond between water and the protein in this region is formed. Since 270 K is the temperature beyond which water in the vicinity of hydrophobic sites becomes mobile, the combination of the results suggests that the dynamic crossover in the water illustrated in the Fig. 2(b) is linked with the unbound of the water molecules shown in the Fig. 4(b). This change in the water dynamics can be observed as a change of curvature in the slowest relaxation protein–water collective motions [50] at temperatures around 270 K.

Next, we check if the temperature dependence of the lifetime of the water–water and water–protein hydrogen bonds is consistent with the mechanisms of water–protein interaction suggested above. The Fig. 5(a) illustrates that the lifetime of the water–water HB are more stable near hydrophobic regions than near the hydrophilic sites, since the former presents a longer lifetime. Around 240 K the lifetime of the water–water bonds close to the hydrophilic sites becomes the same as the value for the bulk water. The Fig. 5(b) shows that the lifetime of the water–protein hydrogen bonds close to the hydrophilic sites are more bounded than the water close to the hydrophobic sites.

This result is consistent with the idea that the hydration water shows a very distinct behavior when confined in globular proteins [51], in hydrophobic [52] and in hydrophilic [53] nanopores due to the dominant interaction with the confining system. While close to the hydrophilic sites the dynamics shows a change at 240 K due to the mobility of the protein–water entity, the water close to the hydrophobic sites becomes more mobile by unbinding from the protein. Our simulations supports that the 240 K transition is related to the kinetics of the water–protein binding and also suggests that the system exhibits a two steps crossover.

4. Summary and conclusions

In this work the water–protein dynamics and the structure were analyzed in the hydrophobic and hydrophilic vicinities by exploring the behavior of water near the TS-Kappa globular protein. The diffusion coefficient of the water close to the hydrophilic sites shows a crossover at 240 K that is the value proposed by experiments and simulations for the dynamical transition of the water–protein system. Close to the hydrophobic sites, however, the crossover is observed for higher temperatures.

The MSD of the protein also shows a crossover at 240 K what suggests that the water–protein dynamics is governed by the water at the hydrophilic sites. This cooperative dynamics is also indicated by the higher $n_{\text{w-pHB}}$ compared with the $n_{\text{w-wHB}}$ about this temperature.

In the case of the water close to the hydrophobic sites the crossover is determined by the unbinding of the water from the protein. Around 270 K the number of water–protein hydrogen bonds becomes zero while the water–water are still bonded.

Our results supports the idea of a common kinetics between water and the protein. This coupling is associated with the functioning of the protein and therefore of relevance for biological, chemical and physical processes.

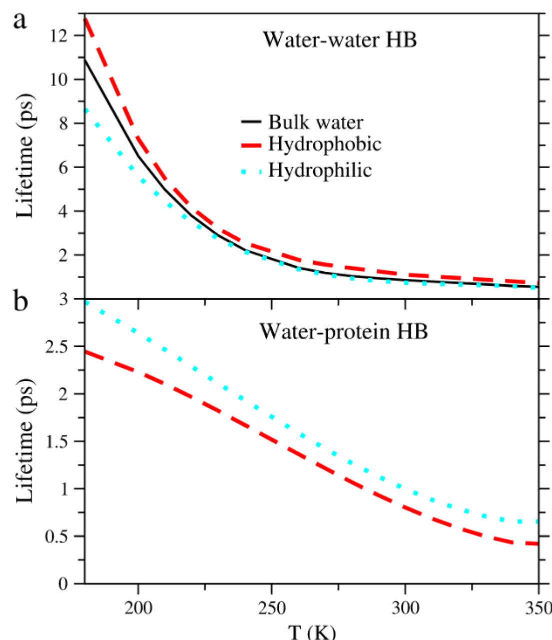

Fig. 5. (a) Lifetime of the water–water HBs at the bulk (solid line), near the hydrophilic (dotted line) and hydrophobic (dashed line) sites of the protein. (b) Lifetime of the HBs between water and hydrophilic/hydrophobic sites of the protein.

Acknowledgments

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References

[1] M.-C. Bellissent-Funel, A. Hassanali, M. Havenith, R. Henchman, P. Pohl, F. Sterpone, D. van der Spoel, Y. Xu, A.E. Garcia, Water determines the structure and dynamics of proteins, Chem. Rev. 116 (2016) 7673–7697.
[2] P. Ball, Water as an active constituent in cell biology, Chem. Rev. 108 (2008) 74–108.
[3] W. Doster, S. Cusack, W. Petry, Dynamical transition of myoglobin revealed by inelastic neutron scattering, Nature 337 (1989) 754–756.
[4] G. Zaccai, How soft is a protein? A protein dynamics force constant measured by neutron scattering, Science 288 (2000) 1604–1607.
[5] W. Doster, H. Nakagawa, M. Appavou, Scaling analysis of bio-molecular dynamics derived from elastic incoherent neutron scattering experiments, J. Chem. Phys. 139 (2013) 045105.
[6] U. Lehnert, V. Reat, M. Weik, G. Zaccai, C. Pfister, Thermal motions in bacteriorhodopsin at different hydration levels studied by neutron scattering: correlation with kinetics and light-induced conformational changes, Biophys. J. 75 (1998) 1945–1952.
[7] S. Kim, D. Gupta, P. Debenedetti, Computational investigation of dynamical transitions in trp-cage miniprotein powders, Sci. Rep. 6 (2016) 25612.
[8] J.A. Rupley, G. Careri, Protein Hydration and Function., Vol. 41, Academic Press, San Diego, CA, 1991, pp. 37–161.
[9] A. Ostermann, R. Waschipky, F.G. Parak, G.U. Nienhaus, Ligand binding and conformational motions in myoglobin, Nature 404 (2000) 205–208.
[10] W. Doster, M. Settles, Protein-water displacement distributions, Biochim. Biophys. Acta - Proteins Proteomics 1749 (2005) 173–186.
[11] G. Schirò, Y. Fichou, F.-X. Gallat, K. Wood, F. Gabel, M. Moulin, M. Hartlein, M. Heyden, J.-P. Colletier, A. Orecchini, A. Paciaroni, J. Wuttke, D. Tobias, M. Weik, Translational diffusion of hydration water correlates with functional motions in folded and intrinsically disordered proteins, Nature Commun. 6 (2015) 6490.
[12] A. Tsai, D. Neumann, L. Bell, Molecular dynamics of solid-state lysozyme as affected by glycerol and water: A neutron scattering study, Biophys. J. 79 (2000) 2728–2732.
[13] J.-M. Zanotti, G. Gibrat, M.-C. Bellissent-Funel, Hydration water rotational motion as a source of configurational entropy driving protein dynamics. crossovers at 150 and 220 k, Phys. Chem. Chem. Phys. 10 (2008) 4865–4870.
[14] K. Wood, A. Frolich, A. Paciaroni, M. Moulin, M. Hartlein, G. Zaccai, D.J. Tobias, M. Weik, Coincidence of dynamical transitions in a soluble protein and its hydration water: direct measurements by neutron scattering and md simulations, J. Am. Chem. Soc. 130 (14) (2008) 4586–4587.
[15] S.-H. Chen, L. Liu, E. Fratini, P. Baglioni, A. Faraone, E. Mamontov, Observation of fragile-to-strong dynamic crossover in protein hydration water, Proc. Natl. Acad. Sci. USA 103 (2006) 9012–9016.
[16] P. Kumar, Z. Yan, L. Xu, M. Mazza, S. Buldyrev, S.-H. Chen, S. Sastry, H. Stanley, Glass transition in biomolecules and the liquid-liquid critical point of water, Phys. Rev. Lett. 97 (2006) 177802.
[17] G. Schirò, M. Fomina, A. Cupane, Protein dynamical transition vs. liquid-liquid phase transition in protein hydration water, J. Chem. Phys. 139 (2013) 121102.
[18] N.V. Nucci, M.S. Pometun, A.J. Wand, Site-resolved measurement of water-protein interactions by solution nmr, Nat. Struct. Mol. Biol. 18 (2011) 245–249.
[19] S. Combet, J.-M. Zanotti, Further evidence that interfacial water is the main driving force of protein dynamics: a neutron scattering study on perdeuterated c-phycocyanin, Phys. Chem. Chem. Phys. 14 (2012) 4927–4934.
[20] Y. Fichou, G. Schirò, F.-X. Gallat, C. Laguri, M. Moulind, J. Combet, M. Zamponi, Hydration water mobility is enhanced around tau amyloid fibers, Proc. Natl. Acad. Sci. USA 112 (2015) 6365–6370.
[21] F. Mallamace, C. Corsaro, D. Mallamace, S. Vasi, C. Vasi, H.E. Stanley, S.-H. Chen, Some thermodynamical aspects of protein hydration water, J. Chem. Phys. 142 (2015) 215103.
[22] A. Pavlova, C.-Y. Chenga, M. Kinnebrew, J. Lew, F. Dahlquist, S. Han, Protein structural and surface water rearrangement constitute major events in the earliest aggregation stages of tau, Proc. Natl. Acad. Sci. USA 113 (2) (2016) 127–136.

[23] M.A. Donovan, Y. Yimer, J. Pfaendtner, E. Backus, M. Bonn, T. Weidner, Ultrafast reorientational dynamics of leucine at the air-water interface, J. Am. Chem. Soc. 138 (2016) 5226–5229.
[24] F. Mallamace, C. Corsaro, D. Mallamace, S. Vasi, C. Vasi, P. Baglioni, S.V. Buldyrev, S.-H. Chen, H.E. Stanley, Energy landscape in protein folding and unfolding, Proc. Natl. Acad. Sci. USA 113 (2016) 3159–3163.
[25] A.R. Bizzarri, S. Cannistraro, Molecular dynamics of water at the protein-solvent interface, J. Phys. Chem. B 106 (2002) 6617–6633.
[26] F. Pizzitutti, M. Marchi, F. Sterpone, P.J. Rossky, How protein surfaces induce anomalous dynamics of hydration water, J. Phys. Chem. B 111 (2007) 7584–7590.
[27] E. Blanc, C. Lecomte, J.V. Rietschoten, J.M. Sabatier, H. Darbon, Solution structure of tskapa, a charybdotoxin-like scorpion toxin from tityus serrulatus with high affinity for apamin-sensitive ca^{2+}-activated k^{+} channels, Proteins 29 (3) (1997) 359–369.
[28] H.J.C. Berendsen, J.R. Grigera, T.P. Straatsma, The missing term in effective pair potentials, J. Phys. Chem. 91 (1987) 6269–6271.
[29] B. Hess, C. Kutzner, D. van der Spoel, E. Lindahl, Gromacs 4: Algorithms for highly efficient, load-balanced, and scalable molecular simulation, J. Chem. Theory Comput. 4 (2008) 435–447.
[30] P. Rani, P. Biswas, Diffusion of hydration water around intrinsically disordered proteins, J. Phys. Chem. B 119 (42) (2015) 13262–13270.
[31] A.C. Fogarty, D. Laage, Water dynamics in protein hydration shells: The molecular origins of the dynamical perturbation, J. Phys. Chem. B 118 (2014) 7715–7729.
[32] W.L. Jorgensen, J. Tirado-Rives, The opls (optimized potentials for liquid simulations) potential functions for proteins, energy minimizations for crystals of cyclic peptides and crambin, J. Am. Chem. Soc. 110 (1988) 1657–1666.
[33] H.M. Berman, et al., The protein data bank, Nucleic Acids Res. 28 (2000) 235–242. (www.rcsb.org).
[34] J.-P. Ryckaert, G. Ciccotti, H.J.C. Berendsen, Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes, J. Comput. Phys. 23 (1977) 327–341.
[35] M. Parrinello, A. Rahman, Polymorphic transitions in single crystals: A new molecular dynamics method, J. Appl. Phys. 52 (1981) 7182.
[36] W.G. Hoover, Canonical dynamics: Equilibrium phase-space distributions, Phys. Rev. A 31 (1985) 1695.
[37] W.G. Hoover, Constant-pressure equations of motion, Phys. Rev. A 34 (1986) 2499.
[38] D. van der Spoel, et al., Thermodynamics of hydrogen bonding in hydrophilic and hydrophobic media, J. Phys. Chem. B 110 (9) (2006) 4393–4398.
[39] C. Rocchi, A.R. Bizzarri, S. Cannistraro, Water dynamical anomalies evidenced by molecular-dynamics simulations at the solvent-protein interface, Phys. Rev. E 57 (1998) 3315–3325.
[40] M. Marchi, F. Sterpone, M. Ceccarelli, Water rotational relaxation and diffusion in hydrated lysozyme, J. Am. Chem. Soc. 124 (23) (2002) 6787–6791.
[41] R. Abseher, H. Schreiber, O. Steinhauser, The influence of a protein on water dynamics in its vicinity investigated by molecular dynamics simulation, Proteins: Struct., Funct., Bioinf. 25 (1996) 366–378.
[42] M.P. Allen, D.J. Tildesley, Computer Simulation of Liquids, Clarendon, Oxford, 1987.
[43] B. Bagchi, Water dynamics in the hydration layer around proteins and micelles, Chem. Rev. 105 (9) (2005) 3197–3219.
[44] J. Qvist, E. Persson, C. Mattea, B. Halle, Time scales of water dynamics at biological interfaces: peptides, proteins and cells, Faraday Discuss. 141 (2009) 131–144.
[45] P. Netz, F. Starr, M.C. Barbosa, H. Stanley, Relation between structural and dynamical anomalies in supercooled water, Physica A 314 (2002) 470–476.
[46] P. Netz, F. Starr, M.C. Barbosa, H.E. Stanley, Computer simulation of dynamical anomalies in stretched water, Braz. J. Phys. 34 (2004) 24.
[47] M.G. Mazza, K. Stokelya, S.E. Pagnotta, F. Bruni, H.E. Stanley, G. Franzese, More than one dynamic crossover in protein hydration water, Proc. Natl. Acad. Sci. USA 111 (50) (2011) 19873–19878.
[48] W. Doster, S. Busch, A.M. Gaspar, M.-S. Appavou, J. Wuttke, H. Scheer, Dynamical transition of protein-hydration water, Phys. Rev. Lett. 104 (2010) 098101.
[49] P.W. Fenimore, Bulk-solvent and hydration shell fluctuations, similar to α- and β-fluctuations in glasses, control protein motions and functions, Proc. Natl. Acad. Sci. USA 101 (2004) 14408–14413.
[50] M. Wolf, S. Emmert, R. Gulich, P. Lunkenheimer, A. Loidl, Dynamics of protein hydration water, Phys. Rev. E 92 (2015) 032727.
[51] R.C. Barbosa, M.C. Barbosa, Hydration shell of the ts-kappa protein: higher density than bulk water, Physica A 439 (2015) 48–58.
[52] M.H. Köhler, L.B. da Silva, Size effects and the role of density on the viscosity of water confined in carbon nanotubes, Chem. Phys. Lett. 645 (2016) 38–41.
[53] L.B. Krott, M.C. Barbosa, Model of waterlike fluid under confinement for hydrophobic and hydrophilic particle-plate interaction potentials, Phys. Rev. E 89 (2014) 012110.