Gen. Physiol. Biophys. (2009), **28**, 168–173

doi:10.4149/gpb_2009_02_168

Dynamics of hydration water in proteins

José Teixeira

Laboratoire Léon Brillouin (CEA/CNRS), CEA Saclay, Gif-sur-Yvette Cedex, France

Abstract. Thermodynamic and transport properties of liquid water are determined essentially by inter-molecular hydrogen bonds and their dynamics. Because the molecular dynamics depends mostly on geometric constrains and dynamics of hydrogen bonds, it is shown that a simple statistics on the number of bonds, including at the vicinity of hydrophilic substrates describes well the temperature dependence of water diffusion on the surface of a small peptide.

Experiments were performed by incoherent quasi-elastic neutron scattering giving information about the hydrogen bond lifetime and the residence time of the water molecules. In order to study the interactions between water molecules and a small hydrophobic peptide (an assembly of five monomers of alanine), the hydration level is changed step by step. The only motion of the first single water molecule of the structure is due to hydrogen libration. The first two added water molecules bind with the hydrophilic site of the peptide and have only the dynamics of the bond breaking. The other hydration molecules show fast diffusive motions on the surface of the peptide.

Key words: Water — Dynamics — Hydrogen bonds — Hydration — Neutron scattering

Introduction

Among the unsolved problems of physics, that of the properties of liquid water is one of the more important, not only because of its fundamental aspects but also because of its impact in many domain of research.

In this short contribution, we address some problems of hydration in biophysics which, more generally, correspond to situations of confinement and interfaces. The knowledge of water properties in such conditions is of crucial importance because, very often, it determines the details of biological activity. For example, it was shown that a minimum amount of water (of the order of 3%) is necessary to the activity of proteins and that this amount of water corresponds to the percolation of hydrogen bonds in a first hydration layer (Careri et al. 1986). More generally, it is a common statement to say that liquid water is a necessary condition for live what, incidentally, implies that only planets where the pressure-temperature conditions fall in the narrow region around the so called "room conditions" may provide life conditions.

The water molecule is a simple molecule as it is formed by three atoms of two different species, one oxygen and two hydrogens forming a V-shape. The complexity arrives from the interaction between neighbouring water molecules. Because of an anisotropic intra-molecular charge distribution and the existence of lone pairs, hydrogen bonds form very easily in a way that a hydrogen atom sits asymmetrically between two oxygens. In a similar way, hydrogen bonds can be formed between a water molecule and other atoms, such as silica (through silanol groups), carboxyl groups, etc. The second important property to quote is the numerical value of the intra-molecular angle H-O-H, which is a consequence of the orbital hybridising of oxygen and hydrogen atoms. Its value is almost equal to that formed by the diagonals of a cube ($\cos^{-1}(-1/3) = 109°$), what generates an almost perfect and extended tetrahedral network.

The third property is more subtle because it concerns the dynamics of the bonds, which is very short (of the order of 1 ps). Actually, the energy of a hydrogen bond is relatively high because it represents 4 or 5 times the thermal energy at room temperature, meaning that water should be a gel! However, the attractive potential due to hydrogen bonds is very directional with high absolute value only in narrow regions around the OH...O alignment. In practice, it is assumed that a bond is "intact" if the hydrogen atom doesn't go out of a 30° cone-centred at the oxygen atom. But the libration motions of the hydrogen bring it out of this cone very easily because the atom is very light and because the low

Correspondence to: José Teixeira, Laboratoire Léon Brillouin, CEA Saclay, F-91191 Gif-sur-Yvette Cedex, France
E-mail: jose.teixeira@cea.fr

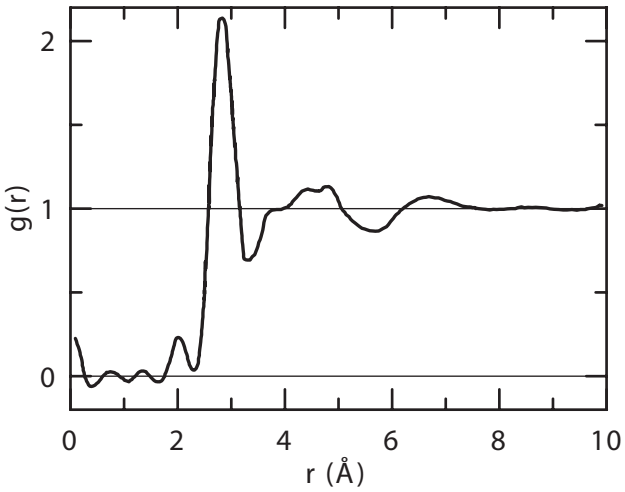

Figure 1. Pair correlation function (g(r)) of D_2O, determined by X-ray scattering at 11.2°C (temperature of the maximum density). The function is proportional to the number of molecules (oxygen atoms) at a distance r from a molecule at the origin (Bosio et al. 1983).

coordination of the molecules generates a large free volume. Statistically, the hydrogen atom goes out of the bond alignment, i.e. the bond "breaks" every picosecond. It is worth noting that libration motions are pure quantum zero point motions. Consequently, the fluidity of water is due to such quantum effects.

The behaviour of water is almost entirely defined by the spatial and temporal properties of each bond yielding a large variety of situations. For example, around hydrophobic solutes, water forms short-lived cages similar to the polyedra that constitute clathrates. Along an extended hydrophobic surface, a layer of vapour is formed in order to favour a planar arrangement of bonds. Instead, around an ion, a relatively small number of water molecules adopt an orientation with the dipole directed in the direction of the electrical charge, screening the associated local electrical field. In some cases of hydrophilic substrates, the bond between a given water molecule and an atom of the surface can be extremely stable.

Because of a so large variety of situations, it is impossible to develop a general theory of water solutions or colloidal suspensions containing water. However, some general trends can be put forward in several situations of confinement.

In the next paragraph, we describe a simple way of computing the connectivity properties of the hydrogen bond network in bulk water and at the vicinity of simple substrates. The third paragraph will present some results concerning the behaviour of water molecules hydrating proteins or peptides. The last paragraph summarizes the main results and discusses more difficult and open issues.

Dynamics of water molecules

One of the more remarkable features of liquid water is the high level of order of the first neighbours shell and the small long range order beyond this distance. The first is due to the high directionality of the intermolecular bonds which imposes an almost perfect local tetrahedral structure within the first neighbour's shell while the resultant small coordinance (between 4 and 5) allows a very high flexibility of the O-O-O angles what spreads the position of the second neighbour in the pair correlation function of the oxygen atoms (Figure 1). Despite the absence of long range order, the number of "intact" bonds at a given time is always very high and beyond the percolation threshold (Geiger et al. 1979). In other words, at short times of observation (of the order of a few picoseconds) water is a gel. Then, it is possible to focus the attention on the bonds, their spatial arrangement and statistics. This idea was developed in Stanley and Teixeira (1980) and it could be established that each water molecule can be classified within one of five classes depending on the number of intact bonds (0 to 5) that it forms with its 4 next neighbours. This picture is oversimplified, at least because the number of next neighbours is, in average, larger than 4, particularly at high temperatures, but it is remarkably correct in the prediction of the population (f_i) of each class of molecules, which follows simply the binomial distribution:

$$f_i = \binom{4}{i} p_{HB}^i (1 - p_{HB})^{4-i}$$

where p_{HB} represents the probability of bond formation, directly related to the number of intact bonds per molecule (n_{HB}) at a given temperature. The fitting of several properties shows that, within the relatively small range of the liquid state, p_{HB} is given by:

$$p_{HB} = n_{HB} / 4 = 1.8 - 0.004T$$

As noticed above, the lifetime of each bond in bulk water (τ_{HB}) is very short and depends weakly on temperature T. Incoherent quasi-elastic neutron scattering (QENS) (Teixeira et al. 1985) and depolarized light scattering (Conde and Teixeira 1984) allow its evaluation at different temperatures. As one may expect, it follows an Arrhenius law

$$\tau_{HB}/\text{ps} = 0.0485\exp(E_{HB}/k_B T)$$

where E_{HB} = 1.85 kcal mol^{-1} is the bond energy. Similarly to the dynamics of side chains in polymers and gels, the hydrogen bond dynamics is the analogous of a β relaxation which, together with the structural constrains generates an α relaxation (τ_{res}), which characterises a residence times determining the dynamics of both molecular rotations and diffusion.

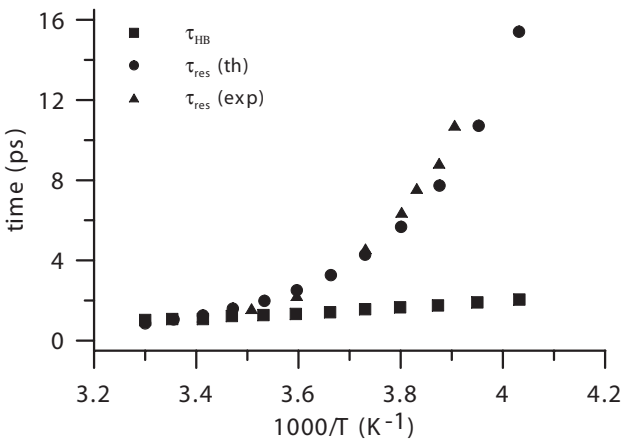

Figure 2. Arrhenius plot of the hydrogen lifetime τ_{HB} (squares) and of the residence time $\tau_{res.}$ (triangles). Both are evaluated experimentally by QENS. The residence time evaluated by percolation concepts is plotted as well (dots). Labels th and exp mean theory and experiment, respectively.

In order to establish a numerical relation between τ_{HB} and τ_{res} it is assumed that molecular motions can be achieved solely by molecules that form not more than one bond with the neighbours. Actually, the main mechanism for rotation in water is indeed rotational jumps around one bond (Ludwig 2007). Consequently, the fraction of immobile molecules is given by $F_I(p_{HB}) = f_2 + f_3 + f_4$, which is a well-defined function of the temperature. τ_{res} can be immediately evaluated as a function of temperature. For this purpose, we consider a given water molecule at time t = 0. Within the assumption of ergodicity, the probability that this molecule remains immobile coincides with $F_I(p_{HB})$, what means that this molecule is part of the ensemble $F_I(p_{HB})$ for the duration τ_{HB}. τ_{res} of the same molecule can be seen as a continuous succession of n successive time steps of duration τ_{HB} : $\tau_{res} = n\ \tau_{HB}$. If the formation of intermolecular bonds is random, i.e. in absence of cooperativity on bond formation, we can write that the probability of τ_{res} for the selected molecule is given by $[F_I(p_{HB})]^n$ that can be identified with a numerical value of the order 1/2. Because p_{HB} is a known function of temperature, we end up with an expression that depends solely on the temperature T:

$$\tau_{res} = Cte \frac{\tau_{HB}(T)}{\left|\ln\left(F_I(T)\right)\right|}$$

where Cte is a constant of the order of unity.

Figure 2 depicts the two times and establishes the comparison with QENS data (Teixeira et al. 1985).

Normally, at the vicinity of a hydrophilic substrate, water makes very stable bonds. This is, for example, the case of silica, because of bridges between silanol groups and water molecules Si-OH...O (Dore 2000). It is worth noting that the stability of such bonds is not necessarily due to a deeper attractive potential but to the fact that libration motions of the hydrogen atoms in the direction perpendicular to the bond may have restricted amplitude of motion what keeps the hydrogen atoms within the region of strong attraction forces.

Within the simple picture presented above for pure water, it is possible to generalise the statistical treatment to the water molecules sitting at the vicinity of the hydrophilic substrate. Assuming that the lifetime of the bond with the hydrophilic substrate is sufficiently stable, the number of short-lived bonds is restricted to 3. The calculation schematised above can be done in a similar way, restricting the number of "breakable" bonds to 3, instead of 4. The subset of molecules immobile at a given time is now $F_I^{'} = f_2^{'} + f_3^{'}$, where the prime means that the binomial distribution is now evaluated for 3 bonds. The resultant molecular dynamics of bonded molecules is given now by:

$$\tau_{res}^{'} = Cte \frac{\tau_{HB}(T)}{\left|\ln\left(F_I^{'}(T)\right)\right|}$$

Figure 3 shows that, as expected, the characteristic time for water molecules forming bonds with a substrate (τ_2), i.e. that are in the first hydration layer is substantially longer compared with the time in bulk water (τ_1). Numerically, $\tau_1 = \tau_{res}$ and $\tau_2 = \tau'_{res}$, if the adjustable constants are equal to unity. In a simplified way, we can say that their dynamics corresponds to that at bulk water at temperatures lower than around 30°C (Teixeira et al. 1997). This statement applies only to the two dimensional diffusion motions on the surface of a hydrophilic substrate. It results from the existence of a stable bond formed between water molecules and the hydrophilic groups (for example, silanol groups on silica). As soon as hydration increases, bonds are formed between water molecules sitting in the first and second hydration layers. Bulk water dynamics is rapidly observed beyond these relatively small hydration levels.

Dynamics of hydration water

The experimental study of the structure and dynamics of water in the vicinity of a large molecule, such as DNA or a protein is a challenge but the importance of the problem justifies a lot of efforts essentially achieved by NMR (Halle and Denisov 1995) and, in a more exhaustive way, by neutron scattering (Ball 2008, and references therein). One of the main problems is the fact that water molecules are structurally in many different situations, as for example, deeply inserted on grooves, forming "bridges" between different residues, or exchanging rapidly with the external aqueous media. The presence of ions and charge variations due to

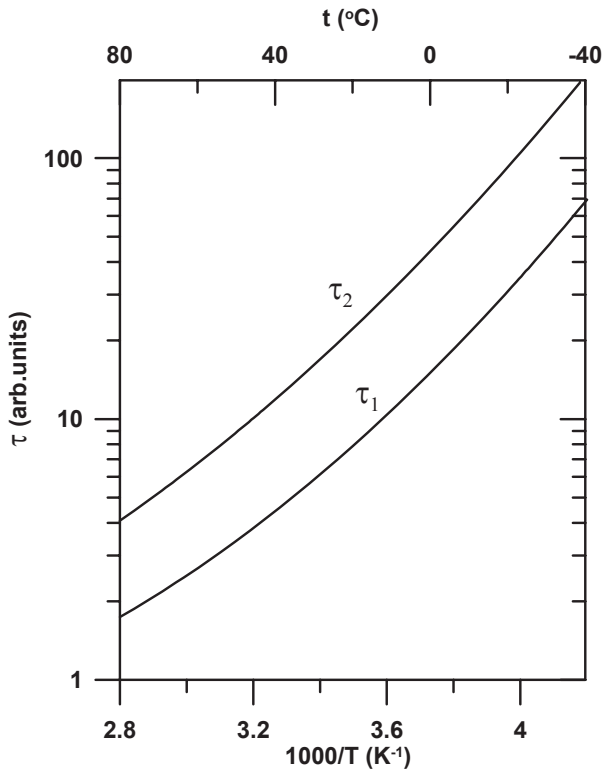

Figure 3. Arrhenius plot of the residence time of water in bulk form (τ_1) and within a hydrophilic confinement (τ_2) (Teixeira et al. 1997).

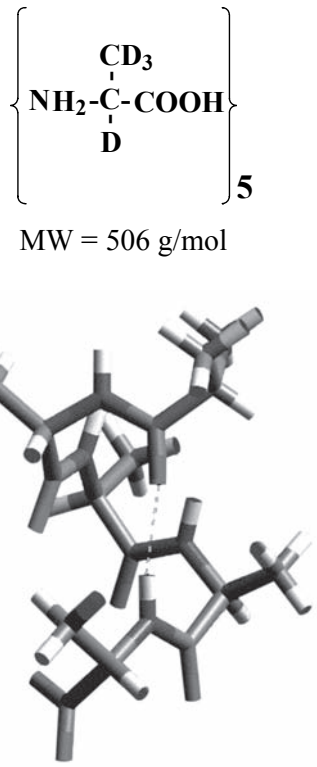

Figure 4. Chemical formula and structural representation of the *ad hoc* peptide formed by 5 monomers of alanine.

pH make the real situation extremely complex and specific of each situation.

Despite these difficulties, some general trends can be established experimentally together with computer molecular dynamics simulations. As an example, we present here the study of the hydration of a peptide *ad hoc*, synthesized in order to represent as well as possible a situation of hydrophobicity.

We synthesized a molecule containing 5 deuterated alanines (Russo et al. 2003), which is hydrophobic due to the methyl (CD_3) groups (Figure 4). Actually, because of the caps necessary to neutralize the charges at the two extremities of the peptide, one single region is hydrophilic. The number of monomers is chosen equal to 5 because it corresponds to the number of monomers in each loop of an α ring of alanine. The sample is prepared as a de-hydrated powder, actually with one stable water molecule forming an internal bond (Figure 4). The experiment consists to measure the dynamics of hydration water by QENS. For this propose, the alanine peptide is full deuterated what means that the scattered intensity is almost totally due to the hydrogen atoms of water surrounding the peptide. Hydration is performed step by step, i.e. at controlled levels of hydration, corresponding to successively, 2, 8, 14 and 25 water molecules per peptide, the last level corresponding to full hydration. Under these conditions, the first two added water molecules form naturally bonds with the only hydrophilic part of the peptide while the next added water molecules don't form any bond but are confined to the surface of the alanine. Figure 5 depicts two representative results of the experiments. For all experiments, the energy dependence of the quasi-elastic scattered intensity is analysed either with one (whenever possible) or two Lorentzians. In every case, the linewidth is clearly independent of the exchange of momentum transfer (Q). It represents large amplitude librations or hindered rotations at the origin of the hydrogen bond breaking and to the time τ_{HB} discussed above. The value of the linewidth corresponds indeed to $\tau_{HB} = (3\Gamma_2)^{-1} \approx 0.9$ ps and is independent of the level of hydration, showing that it represents a fundamental property of hydrogen bonds in water. The smaller width (larger time) observed for the dry protein shows that the water molecule that is part of the structure of the peptide has a much slower dynamics, due to confinement. For hydrations equal or above 30% (8 water molecules per peptide), another, Q-dependent component, must be included in the data fitting. It corresponds to translational diffusion of all the water molecules not attached to the hydrophilic site. It is remarkable that the diffusion, extracted from the initial

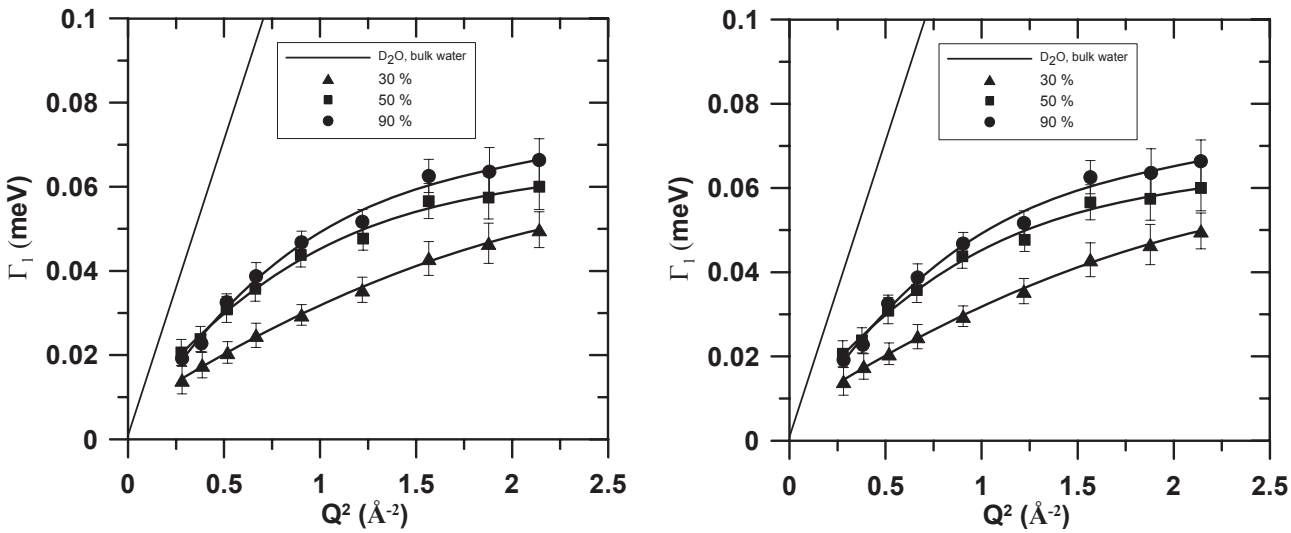

Figure 5. Linewidth of the two Lorentzian lines that fit the QENS data of alanine-5 dry and hydrated at different levels plotted against the momentum transfer Q. Γ_1 is the width of the narrow component which represents translational diffusion (absent in the case of the dry peptide). Γ_2 represents hindered rotations, more exactly, large amplitude librations of the hydrogen atoms across the hydrogen bonds (Russo et al. 2008).

slope of Γ_1 (Q^2) varies from 0.77 to 1.36×10^{-5} cm^2s^{-1}, only a factor 2 to 3 smaller than self-diffusion in bulk water. The residence time, $\tau_{res} = \lim_{Q\to\infty} \Gamma_1^{-1}$, is constant and equal to 7 ps what corresponds to bulk supercooled water at –10°C (Teixeira et al. 1985), what is due to a motion constrained to 2 dimensions.

Conclusion

In this short review we called the attention to the extreme variety of situations of confined water, showing some examples of the behaviour at the vicinity of peptides. Depending on the nature of the interface, water may form more or less stable hydrogen bonds. Their stability (lifetime) can go from a picoseconds to infinity but, in all cases, the characteristic time of large amplitude librations is very short: 1 ps or less. Depending on the local topology and on the intensity of the bonds, the libration motions are more or less effective on hydrogen bond breaking what explains the very large range of the possible residence times. Concerning self-diffusion, it is easier at the vicinity of hydrophobic interfaces. Recent studies have shown that a layer of water vapour exists at the vicinity of hydrophobic surfaces (Mezger et al. 2006) confirming also different results obtained by simulations of the molecular dynamics (Luzar and Leung 2000). Also, it was observed that the dynamics of hydration water is different in the case of peptides that differ only by the presence of a hydrophobic chain in the first case as shown in the cases of N-acetyl-leucine-methylamide and N-acetyl-glycine-methylamide (Russo et al. 2008).

These systematic studies of hydration water molecules both in powders and in solution throw light on the role of water in biological processes.

References

Ball P. (2008): Water as an active constituent in cell biology. Chem. Rev. **108,** 74–108; doi:10.1021/cr068037a PMid:18095715

Bosio L., Chen S.-H., Teixeira J. (1983): Isochoric temperature differential of the X-ray structure factor and structural rearrangements in low temperature heavy water. Phys. Rev., A **27,** 1468–1475; doi:10.1103/PhysRevA.27.1468

Careri G., Giansanti A., Rupley J. A. (1986): Proton percolation on hydrated lysozyme powders. Proc. Natl. Acad. Sci. U.S.A. **83,** 6810–6814; doi:10.1073/pnas.83.18.6810

Conde O., Teixeira J. (1984): Depolarized light scattering of heavy water and hydrogen bond dynamics. Mol. Physiol. **53,** 951–959; doi:10.1080/00268978400102761

Dore J. C. (2000): Structural studies of water in confined geometry by neutron diffraction. Chem. Phys. **258,** 327–347; doi:10.1016/S0301-0104(00)00208-1

Geiger A., Stillinger F. H., Rahman A. (1979): Aspects of the percolation process for hydrogen-bond networks in water. J. Chem. Phys. **70,** 4185–4193; doi:10.1063/1.438042

Halle B., Denisov V. P. (1995): A nex view of water dynamics in immobilized proteins. Biophys. J. **69,** 242–249;

doi:10.1016/S0006-3495(95)79895-2 PMid:7669901 PMCid:1236241

Ludwig R. (2007): The mechanism of the molecular reorientation in water. Chemphyschem. **8,** 44–46; doi:10.1002/cphc.200600497 PMid:17131431

Luzar A., Leung K. (2000): Dynamics of capillary evaporation. I. Effect of morphology of hydrophobic surfaces. J. Chem. Physiol. **113,** 5836–5844; doi:10.1063/1.1290478

Mezger M., Reichert H., Schöder S., Okasinski J., Schröder H., Dosch H., Palms D., Ralston J., Honkimäki V. (2006): High-resolution in situ x-ray study of the hydrophobic gap at the water-octadecyl-trichlorosilane interface. Proc. Natl. Acad. Sci. U.S.A. **103,** 18401–18404; doi:10.1073/pnas.0608827103
(See also comments by Ocko B. M., Dhinojwala A. and Daillant J. on the letter by Adelé Poynor et al. in Phys. Rev. Lett. (2006) **97,** 26**6101,** and the reply in Phys. Rev. Lett. (2008) **101,** 039601).

Russo D., Baglioni P., Peroni E., Teixeira J. (2003): Hydration water dynamics of a completely hydrophobic oligopeptide. Chem. Phys. **292,** 235–245; doi:10.1016/S0301-0104(03)00200-3

Russo D., Olivier J., Teixeira J. (2008): Water hydrogen bond analysis on hydrophilic and hydrophobic biomolecule sites. Phys. Chem. Chem. Phys. **10,** 4968–4974; doi:10.1039/b807551b PMid:18688541

Stanley H. E., Teixeira J. (1980): Interpretation of the unusual behaviour of H2O and D2O at low temperatures. Tests of a percolation model. J. Chem. Phys. **73,** 3404–3423; doi:10.1063/1.440538

Teixeira J., Zanotti J.-M., Bellissent-Funel M.-C., Chen S.-H. (1997): Water in confined geometries. Physica B 234-**236,** 370–374; doi:10.1016/S0921-4526(96)00991-X

Teixeira J., Bellissent-Funel M.-C., Chen S.-H., Dianoux A. J. (1985): Experimental determination of the nature of diffusive motions of water molecules at low temperatures. Physiol. Rev., A **31,** 1913–1917

Received: October 1, 2008
Final version accepted: December 16, 2008