



ELSEVIER

Journal of Molecular Liquids 78 (1998) 19–28

journal of
**MOLECULAR
LIQUIDS**

Structure and dynamics of water near hydrophilic surfaces

Marie-Claire Bellissent-Funel

Laboratoire Léon-Brillouin (CEA-CNRS), CEA Saclay,
91191 Gif-sur-Yvette, France

Water is essential for the stability and function of biological macromolecules. In living systems, essential water-related phenomena occur in restricted geometries in cells, and at active sites of proteins and membranes or at their surface. In this review paper, we present the more recent up to date account of the structure and dynamics of water confined in pores of porous glass and at surface of a soluble globular protein. The experimental results obtained by neutron scattering are compared with that of bulk water. Some realistic picture of room temperature interfacial water in terms of some increase of the extension of H-bond network of water as it occurs in supercooled water at a temperature 25 K lower is proposed.

1. LIQUID WATER : A FASCINATING TRANSIENT H-BOND GEL

At the microscopic level, liquid water can be seen as a transient gel or as a network of hydrogen bonds with a *local tetrahedral symmetry*. This picture has been developed by the percolation model of Stanley and Teixeira [1] and the connectivity properties studied by computer molecular dynamic (CMD) simulations [2]. From these simulations, it appears that the hydrogen-bond network includes tiny spatially-correlated ‘*patches*’ of four-bonded molecules and that the local density near a patch is lower than the global density. The value of the mean characteristic length is of the order of the correlation length of the density fluctuation as measured both by X-ray scattering [3] and neutron scattering [4]. Within the Ornstein-Zernike theory, the correlation length has been estimated as equal to 8 Å at -20 °C, and it increases when the temperature decreases. © 1998 Elsevier Science B.V. All rights reserved.

1.1. Microscopic structure

The microscopic structure of bulk and confined water is currently studied by using X-ray or /and neutron diffraction techniques which are complementary techniques. These diffraction techniques allow us to access the intermolecular pair correlation function $g(r)$ or $d(r) = 4\pi\rho r[g(r) - 1]$ of a system, where ρ is the molecular number density, that is the probability density of finding another atom lying in another molecule at a distance r from any atom. In X-ray measurements $g(r)$ is the pair correlation function of the molecular centres, to a good approximation equal to the oxygen-oxygen partial correlation function. In neutron measurements $g(r)$ is the weighted sum of the three partial functions relative respectively to

the oxygen-oxygen pairs, oxygen-deuterium pairs and deuterium - deuterium pairs. In particular, it is heavily weighted toward deuterium - deuterium and oxygen-deuterium partial correlation functions.

In most circumstances such as crystalline ice [5], amorphous ice [6], water under pressure [7], with the exception of supercritical water [8], one observes that the coordination number of water is around 4. This is a small number, meaning that there is a large amount of "free space" available for movements such as O-O-O bending. This is apparent on the pair correlation function $d(r)$ (fig. 1), which shows a well defined first peak at 2.8 Å, corresponding to the first shell of neighbours, a broad second neighbour peak at 4.5 Å and dies rapidly beyond the third neighbour distance [9]. This is in contrast with simple atomic liquids, such as argon, for which long range order is clearly observed.

Figure 2 displays the evolution of the pair correlation function of water as obtained by neutron diffraction and as a function of temperature. In the deeply supercooled state, we observe some increase of the spatial correlations which are associated with the extension of the H-bond network [10]. The structure of water evolves towards that of low density amorphous ice [6].

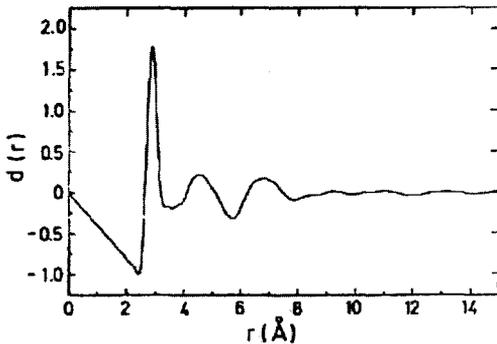


Fig.1. Pair correlation function $d(r)$ of bulk water at room temperature as given by X-ray diffraction.

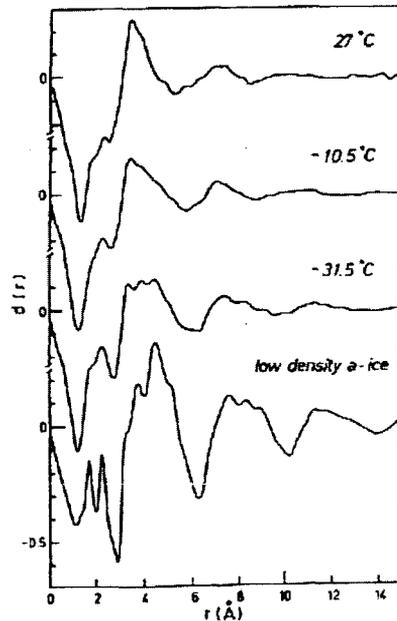


Fig.2. The weighted pair correlation function $d(r)$ of liquid D_2O at different temperatures as determined by neutron scattering; the comparison is done with low density amorphous ice.

1.2. Diffusive motions of water molecules in liquid water

If the local tetrahedral symmetry of water is one of the characteristics of water, a second property is the presence of intermolecular bonds i. e. hydrogen bonds in water. Spectroscopic studies traditionally classify the bonds into two categories: intact and broken, essentially because of the shape of the intramolecular stretching band of the Raman spectrum of liquid water and of its temperature dependence. Such interpretation is questionable but it appears

that a large number of molecules have a strong attractive interaction with their neighbours. One can say that the ensemble of the intact (or with energy beyond some reference energy) bonds constitutes a network well above its percolation threshold. As a consequence, at a given time, essentially all the molecules are part of an "instantaneous gel" [11]. It is worth noting that the two preceding aspects of water structure explain that, in spite of a very short range local order, the connectivity length of the hydrogen bond network is infinite.

However, and this is the third aspect, the characteristic 'life time' of a hydrogen bond is very short: between 10^{-13} and 10^{-12} s and this is why viscoelastic properties of a gel structure will never be observed even in short characteristic time experiments. The explanation of such a short time is that hydrogen bond life times are determined by the proton dynamics [11]. In particular, large amplitude librational movements are responsible for the making and breaking of hydrogen bonds.

This method has been used with success for studying the self dynamics of bulk water as a function of the temperature as previously reported [12], (see figure 3). We have studied by quasi-elastic neutron scattering the individual motions of water molecules in liquid water [13]. This technique is a powerful and unique tool for studying the self dynamics of water; actually the large incoherent scattering cross section of the protons yields unambiguous results about the individual motions of water molecules. In fact, this technique is a method for studying the diffusive motion of atoms in solids and liquids [14]. It gives access to the correlation function for the atomic motions which are explored over a space domain of the order of a few Å and for times of the order of 10^{-12} s. This space and time domain is similar to that of CMD which makes the comparison between neutron scattering and CMD justified. The correlation function can be calculated for various models for the assumed motion of the diffusing particles (e.g., Brownian motion, jump diffusion, diffusion in a confined space, rotational motion,) and the microscopic properties of the environment of the scattering atoms, e. g. the residence time, the jump length, the diffusion coefficients, the H-bond life time become available.

Another quantity that can be obtained is the density of states of mobile protons, in particular the translational and librational modes of water.

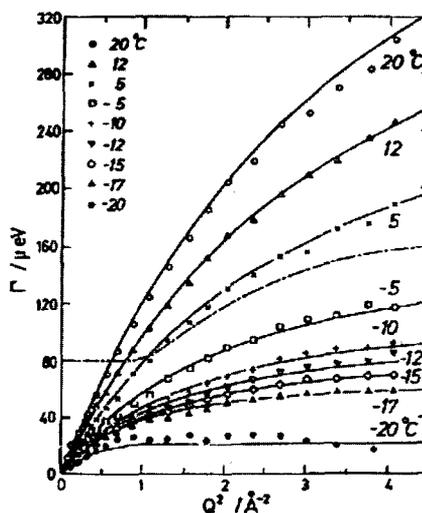


Fig.3. Linewidths of the quasielastic incoherent neutron scattering of bulk water at different temperatures [12]. The plateau at large Q is proportional to the residence time of the molecules. The dashed line represents the Lorentzian linewidth for water bound to C-phycocyanin at room temperature [29].

2. MICROSCOPIC STRUCTURE AND DYNAMICS OF CONFINED WATER/INTERFACIAL WATER

In many technologically important situations, water is not in its bulk form but is instead attached to some substrates, or filling small cavities. Common examples are : water in porous media, such as rock or sand stones and water in biological material as in the interior of cells or attached to surfaces of biological macromolecules and membranes. This is what we define here as the "confined" or the "interfacial water".

Water in confined space has attracted a considerable interest in the recent years. It is commonly believed that the structure and dynamics of water are modified by the presence of solid surfaces, both by a change of hydrogen bonding and by modification of the molecular motion which depends on the distance of water molecules from the surface.

2.1. Water confined in pores of hydrophilic model systems

The structure of confined water has been studied by neutron diffraction. Among the hydrophilic model systems, let us mention clay minerals [15-16], and various types of porous silica [17-18]. In the last case, the authors have interpreted their results in terms of a thin layer of surface water with more extensive H-bonding, lower density and mobility and lower nucleation temperature as compared to bulk water.

The more recent results concern the structure of water confined in a Vycor glass [19] which is a porous silica glass, characterized by a quite sharp distribution of cylindrical interconnected pores, and hydrophilic surfaces. We present here some of our results which have been obtained as functions of the level of hydration from full hydration (0.25g water/g dry vycor) down to 25% and temperature [20-21]. Based on the information that the dry density of Vycor is 1.45 g/ml, the porosity 28% and the internal cylindrical pores of cross sectional diameter 50 Å, the 50% hydrated sample has 3 layers of water molecules on its internal surface and a 25% hydrated sample corresponds roughly to a mono-layer coverage.

Results for two levels of hydration of Vycor demonstrate that the fully hydrated case is almost identical to the bulk water and the partially hydrated case is of little difference (figure 4). However, the three site-site radial correlation functions are

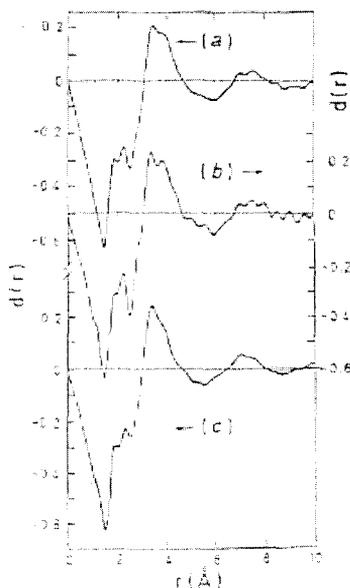


Fig.4. Pair correlation function $d(r)$ for (a) confined D_2O from fully hydrated Vycor (27 °C); (b) confined D_2O from partially hydrated Vycor (35°C) as compared with (c) bulk water (27 °C) [20].

indeed required for a sensible study of the orientational correlations between neighboring molecules

It is interesting, however, to comment on the level of supercooling possible for heavy water in Vycor. According to ref. [20] for partially hydrated samples, the deepest supercooling is -27°C , while for the fully hydrated sample it is -18°C . As the temperature goes below the limit of supercooling, part of the confined water nucleates into cubic ice. The proportion of cubic ice increases with a decreasing temperature. This is in sharp contrast to bulk water which always nucleates into hexagonal ice.

Results relative to a 25% hydrated Vycor sample indicates that, at room temperature, interfacial water has a structure similar to that of bulk supercooled water at a temperature of about 0°C which corresponds to a shift of about 25 K [21]. The structure of interfacial water is characterized by an increase of the long range correlations which correspond to the building of the H-bond network as it appears in low density amorphous ice [22]. There is no evidence of ice formation when the sample is cooled from room temperature down to -196°C (liquid nitrogen temperature).

2.2. Water molecules on surface of a deuterated protein

Let us now introduce a simple picture of a soluble globular protein in terms of a direct micelle with an external hydrophilic surface and an hydrophobic core. The external hydrophilic surface is in contact with water opposite to the hydrophobic core that fearing water is located inside the protein [23-24].

The amount of information about protein-water correlations is small. For neutron diffraction, deuterated samples are required and difficult to obtain. However, the first results have been obtained in the case of a photosynthetic C-phycoyanin protein for which the X-ray crystallographic structure is known to a resolution of 1.66\AA [25].

C-phycoyanin is abundant in blue-green algae. Nearly 99% deuterated samples of this phycobiliprotein were isolated from the cyanobacteria which were grown in perdeuterated cultures [26] (99% pure D_2O) at Argonne National Laboratory. This process yielded deuterated C-phycoyanin proteins (d-CPC) that had virtually all the $^1\text{H-C}$ bonds replaced by $^2\text{H-C}$ bonds. One can obtain a lyophilised sample similar to amorphous solids as determined by neutron diffraction [27]. As has been defined in previous papers [28-29] the level of hydration $h = 0.5$ corresponds to 100% hydration of C-phycoyanin which leads to a coverage of about 1.5 monolayers of water molecules on the surface of the protein.

Recently, the water (D_2O)-protein correlations at the surface of a fully deuterated amorphous protein C-Phycocyanin have been studied by neutron diffraction as functions of temperature and hydration level [27]. The correlation distance of 3.5\AA measured in these diffraction experiments compared well with computer simulation work on polypeptides and proteins [30-31] and has been interpreted as resulting from some increase in the clustering of water molecules (figure 5). For the highest hydrated sample ($h = 0.365$), a definite peak appears at 3.5\AA . This is the average distance between the center of mass of a water molecule in the first hydration layer and amino-acid residues on the surface of the protein. In the case of the lowest hydrated sample ($h = 0.175$), the perturbation to the structure of protein due to water of hydration is not detectable. It is generally viewed in the literature that, at full hydration ($h = 0.5$), there is a complete monolayer of water surrounding the protein [32].

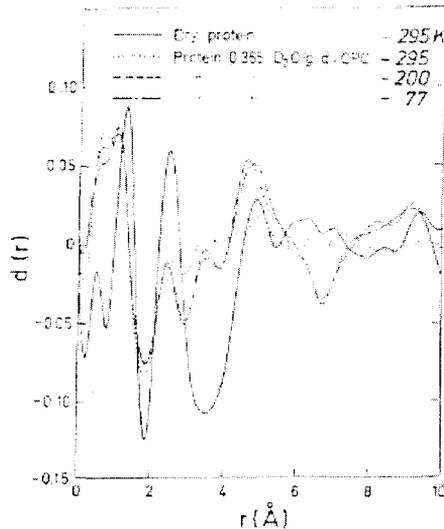


Fig.5. Pair correlation function $d(r)$ for a dry d-CPC protein at 295 K and for a D_2O -hydrated ($h=0.365$) d-CPC protein at different temperatures [27].

A similarity between the behaviour of water close to C-phycoyanin protein and close to hydrophilic model systems can be stressed. In fact, for low hydrated protein samples, no crystallisation of water is detectable while for more than one monolayer coverage there is the appearance of the hexagonal crystalline ice. Moreover, the peak at 3.5 \AA is also detected. However it should be noted that, at the highest hydration level, water nucleates into hexagonal ice at low temperature; in contrast with hydrated Vycor where water nucleates into cubic ice. This difference can be attributed to the presence of rigid walls of Vycor which prevent crystallization into the hexagonal ice phase [20].

The studies of single particle dynamics of hydration water in proteins have been hampered by the fact that about 40% of the constituent atoms in a typical protein molecule are hydrogen atoms, present in the backbone and in the side chains. The elastic contribution is thus too large for an accurate determination of the dynamical parameters characteristic of hydration process. However, by working with a deuterated protein / H_2O system, it has been possible recently to focus on the water dynamics at and near the protein surface [29].

Common features arise from the quasi-elastic neutron scattering studies for water close to some hydrophilic Vycor surface or for water close to a more complex surface such as that of a protein. In the case of water close to different residues of a biological material several contributions have to be considered such as the hydrogen atoms of the protein itself, the possibility of their exchange with water molecules, the presence of hydrophilic and hydrophobic regions.

Results of a C-phycoyanin protein at a hydration level $h = 0.5$ are presented in figure 3. The behaviour of the linewidth Γ of the Lorentzian line as deduced from the quasi-elastic spectra is compared with that of bulk water, at various temperatures. While the linewidth of the bulk water is characteristic of a random jump diffusion model, that of water from hydrated

protein exhibits different behaviour [29]. Γ is analysed at low Q in terms of a model of a diffusion inside a sphere of radius a ; the diffusion coefficient inside the sphere is D_{local} [33]. Similar behaviour is obtained for water confined in the pores of Vycor glass [34]; as the hydration level goes down towards a monolayer coverage, the confinement volume decreases to an equivalent sphere of a radius 3.5 Å and the residence time for jump diffusion increases to more than a factor of ten of the corresponding bulk value [12]. At high- Q , Γ is accounted for by the random jump diffusion model characterized by the diffusion coefficient D_t and the residence times τ_0 .

For hydrated protein, the values obtained for D_{local} , D_t and τ_0 are dependent on the level of hydration. For $h = 0.5$, D_{local} and D_t are similar and D_t is close to that of bulk water; however, it has to be noticed that the residence times τ_0 of confined water from hydrated protein are always longer than the residence time of bulk water at the same temperature.

For $h=0.4$, they are close to those obtained at the same temperature for 25% H₂O-hydrated Vycor [35] which demonstrates the influence of the hydrophilic groups on the water molecules when a monolayer coverage is reached. This shows that the diffusive motion of water molecules is strongly retarded by interactions with a protein surface. The residence times τ_0 of confined water from hydrated Vycor and from hydrated protein are always longer than the residence time of bulk water at the same temperature. They increase rapidly as either the temperature or the level of hydration decreases [35] (Figure 6a).

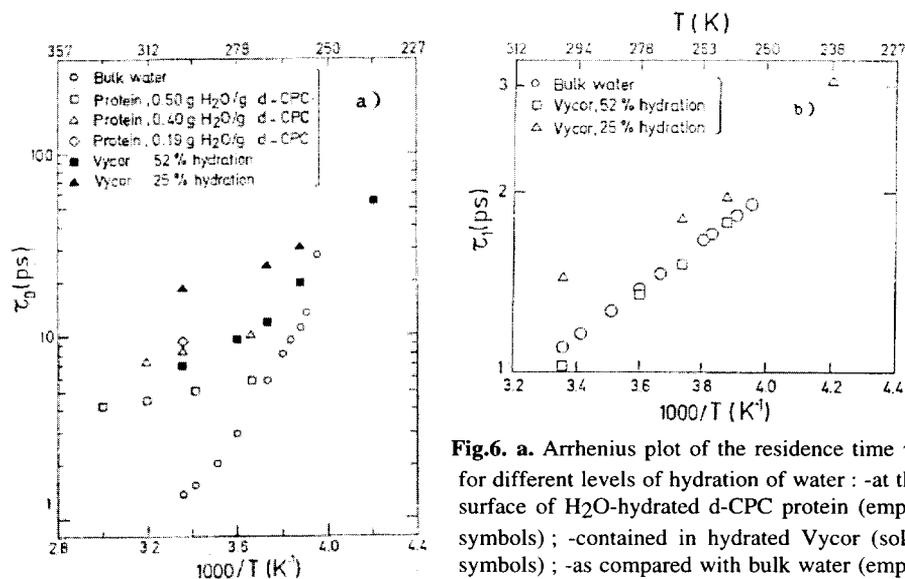


Fig.6. a. Arrhenius plot of the residence time τ_0 for different levels of hydration of water: -at the surface of H₂O-hydrated d-CPC protein (empty symbols); -contained in hydrated Vycor (solid symbols); -as compared with bulk water (empty circles) [35].

b. Arrhenius plot of the hindered rotations characteristic time, τ_1 . This time can be associated with the hydrogen bond life time [34].

For the higher levels of hydration, the values of the hydrogen-bond life time τ_1 for confined water are close to that of bulk water [12] ; when decreasing the level of hydration, the times τ_1 become longer (Figure 6b). This is in good agreement with the shift to higher frequencies of the librational band as it appears in figure 7. In fact the librational modes that are responsible on the making and breaking of the hydrogen bond become hindered in case of interfacial water. However, the hydrogen-bond life time τ_1 for confined water has an Arrhenius temperature dependence while the residence time τ_0 does not exhibit such behaviour (Figures 6a and b).

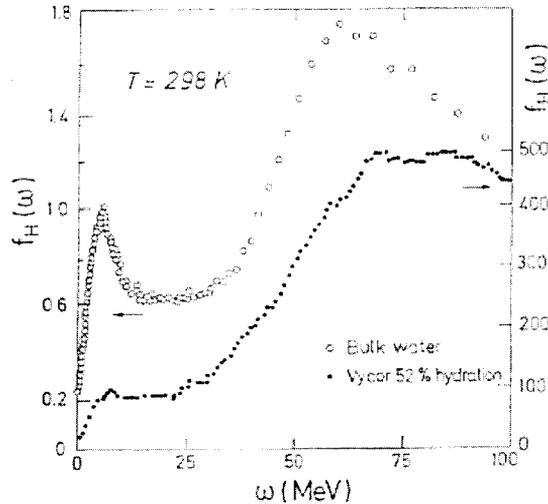


Fig.7. Proton vibrational density of states $f_H(\omega)$ for water contained in 52% hydrated Vycor at 298 K (solid circles). For comparison the corresponding quantity for bulk water (empty circles) is also given [34].

3. DISCUSSION AND CONCLUSION

In this review paper, we have reported on the microscopic structure and the short time translational diffusion (less than 40 picoseconds) of water molecules in micro-pores of hydrated Vycor and on the surface of a hydrated protein as functions of hydration level and temperature. In particular, the analysis of short time dynamics has been done in terms of a simple model. The main conclusion derived from this analysis is that the translational motion of water molecules close to a hydrophilic surface at short time (or equivalently, at large Q), is well characterized by a jump diffusion model, having about the same diffusion constant as the corresponding bulk water but with a considerably slower jump rate.

In a previous paper [36], we pointed out that the slow dynamics can be interpreted in an alternative way. Our more recent fitting approaches of the quasi-elastic spectra relative to 52 % hydrated Vycor (containing 0.13 g water per g of Vycor, i.e. three layers of coverage of water molecules on the hydrophilic interior surface) indicate that the kinetic glass transition described by stretched exponentials is a good method for describing the dynamics of

water confined in porous silica. The slow dynamics is characterized by an α -relaxation with a structural relaxation time that increases from 5 ps at 298 K to 19 ps at 268 K (at $Q = 0.986 \text{ \AA}^{-1}$).

The fitting approaches have been obtained by computer simulations, using a SPCE (Extended Simple Point Charge Model). It has been clearly shown that, upon supercooling, bulk water develops a slow dynamics characterized by a stretched exponential decay of the intermediate scattering function [37]. In order to confirm these results new experiments in the very deeply supercooled state of water have been done, and the analyses are now in progress.

To conclude it is important to stress that the microscopic structure and the dynamics of confined water is similar to that of bulk supercooled water. Considering the similarity between the dynamics of water in the interior of the surfaces of Vycor, and on the surface of protein, and also the fact that they both exhibit slow dynamics we can infer that water near hydrophilic surfaces is effectively at a temperature equivalent to supercooled water at a considerable lower temperature. The typical temperature shift is of the order of 30 K for interfacial water at room temperature and decreases slowly with decreasing temperature [38]. This has a significant biological implication : the existence of the slow dynamics in hydration water of protein at room temperature may be the molecular mechanism that leads to slow dynamics of protein and the consequent anharmonicity required for it to function as an enzyme [32].

ACKNOWLEDGEMENTS

We are grateful to H. L. Crespi for his continuing collaboration in the preparation of the perdeuterated protein C-phycoyanin.

REFERENCES

1. H.E. Stanley and J. Teixeira, *J. Chem. Phys.*, 73 (1980) 3404.
2. A. Geiger and H.E. Stanley, *Phys. Rev. Lett.*, 49 (1982) 1749.
3. L. Bosio, J. Teixeira and H. E. Stanley, *Phys. Rev. Lett.*, 46 (1981) 597
4. L. Bosio, J. Teixeira and M.-C. Bellissent-Funel, *Phys. Rev. A*, 39 (1989) 6612
5. P. V. Hobbs, in "Ice Physics", (Clarendon Oxford) 1974
6. M.-C. Bellissent-Funel, J. Teixeira, L. Bosio, *J. Chem. Phys.*, 87 (1987) 2231.
7. M.-C. Bellissent-Funel and L. Bosio, *J. Chem. Phys.*, 102 (1995) 3727.
8. M.-C. Bellissent-Funel., T. Tassaing, H. Zao, D. Beysens, B. Guillot and Y. Guissani, *J. Chem. Phys.*, 107 (1997) 2942
9. S.H. Chen and J. Teixeira, *Adv. Chem. Phys.*, 64 (1985) 1.
10. M.-C. Bellissent-Funel, in "Hydrogen Bonded Liquids", J.C. Dore and J. Teixeira eds. NATO ASI Series C, Kluwer Academic Publishers, 1991.
11. J. Teixeira, *J. Phys. IV*, 3, C1 (1993) 163 and references therein.
12. J. Teixeira, M.-C. Bellissent-Funel, S.H. Chen and A.J. Dianoux, *Phys. Rev.*, A31 (1985) 1913.
13. S.W. Lovesey, *Theory of neutron scattering from condensed matter*, 3rd edition, Clarendon Press, Oxford, 1987.
14. T. Springer, *Quasi-elastic neutron scattering for the investigation of diffusive motions in solids and liquids* Springer Ser. Modern Phys., 64 (1972).

15. R.K. Hawkins and P.A. Egelstaff, *Clays and Clay Minerals* 28 (1980) 19.
16. A.K. Soper, in "Hydrogen-Bonded Liquids", Eds. J.C. Dore and J. Teixeira, Kluwer Academic Pubs. 329 (1991) 147.
17. J.C.Dore, F. Coveney and M.C. Bellissent-Funel, in "Recent developments in the Physics of Fluids", Eds. W.S. Howells and K. Soper, Adam Hilger Pubs., p.299, 1992.
18. M.J. Benham, J.C. Cook, J.C. Li, A.D.K. Ross, P.L. Hall and B. Sarkissian, *Phys. Rev. B* 39 (1989) 633.
19. General information on Vycor Brand Porous "thirsty glass, n° 7930, Corning Glass Works, is available from OEM Sales Service, Box 5000, Corning, NY 14830, USA .
20. M.-C. Bellissent-Funel, L. Bosio and J. Lal, *J. Chem. Phys.* 98 (1993) 4246.
21. J.-M. Zanotti, Ph D Thesis, 1997.
22. M.-C. Bellissent-Funel, L. Bosio, A. Hallbrucker, E. Mayer and R. Sridi-Dorbez, *J.Chem. Phys.*, 97 (1992) 1282.
23. P.J. Steinbach and B.R. Brooks, *Proc. Natl. Acad. Sci. USA*, 90 (1993) 9135.
24. V. Lounnas and B.M. Pettitt, *Proteins : Structure, Function and Genetics*, 18 (1994) 148.
25. M. Duerring, G.B. Schmidt and R. Huber, *J. Mol. Biol.*, 217 (1987) 577.
26. H.L. Crespi, in *Stable Isotopes in the Life Science ; IAEA, Vienna*, 111, 1977
27. M.-C. Bellissent-Funel, J. Lal, K.F. Bradley and S.H. Chen, *Biophys. J.*, 64 (1993) 1542.
28. S.H. Chen, and M.-C. Bellissent-Funel, in "Hydrogen Bond Networks", M.C. Bellissent-Funel and J.C. Dore (Eds), NATO ASI C435, p. 307, Kluwer Academic Pub., 1994
29. M.-C. Bellissent-Funel, J. Teixeira, K. F. Bradley and S. H. Chen, *J. Phys. I, France*, 2 (1992) 995.
30. B. Lee and F.M. Richard, *J. Mol. Biol.*, 55 (1971) 379.
31. M. Levitt and R. Sharon, *Proc. Natl. Acad. Sci. USA : Biophysics*, 85 (1988) 7557.
32. J.A. Rupley and G. Careri, *Adv. Prot. Chem.*, 41 (1991) 37.
33. F. Volino and A. J. Dianoux, *Mol. Phys.*, 41 (1980) 271.
34. M.-C. Bellissent-Funel, S.H. Chen and J.-M. Zanotti, *Phys. Rev. E*, 51 (1995) 4558
35. M.-C. Bellissent-Funel, J.-M. Zanotti and S.H. Chen, *Far. Disc.* 103 (1996) 281
36. S.H. Chen, P. Gallo and M.-C. Bellissent-Funel, *Non Equilibrium Phenomena in Supercooled Fluids, Glasses and Materials*, World Scientific Publication, (1996) 186
37. P. Gallo, F. Sciortino, P. Tartaglia and S.H. Chen, *Phys. Rev. Lett*, 76 (1996) 2730
38. J. Teixeira, J.-M. Zanotti, M.-C. Bellissent-Funel and S.H. Chen and, *Physica B*, 234-236 (1997) 370.