

## Glass transition in protein hydration water

Michel Peyrard

*Laboratoire de Physique, Ecole Normale Supérieure de Lyon, 46 allée d'Italie, 69364 Lyon Cedex 07, France*

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A model of two-dimensional water is studied in order to analyze recent dielectric measurements on protein hydration water. The frustration introduced by the coupling of the water molecules to the protein surface prevents a true crystallization and induces a smooth transition to a glassy state that is detected by its structure factor and the stretched exponential behavior of the cage correlation function that measures the rate of change of the hydrogen bond network around each molecule. The spectrum of the fluctuations in the vicinity of the glass transition exhibits  $1/f^\alpha$  noise in agreement with the dielectric measurements. The results suggest that two-dimensional water, which can be probed by dielectric measurements, could be an interesting system to study the glassy behavior of water.

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### I. INTRODUCTION

Water is essential for the stability and function of biological molecules and its importance in physics and chemistry cannot be underestimated. However its properties are still far from being completely understood. There has been a recent renewal of interest in its dynamics and thermodynamics [1], and the results support the hypothesized continuity of dynamic functions between the liquid and glassy states of water. While experimental studies of the glassy behavior of bulk water are extremely difficult, hydration water, in particular water on the surface of proteins, can provide an interesting alternative to study the slow dynamics water because, in this case, the binding of the molecules to a substrate can prevent crystallization. Slow dynamics of protein interfacial water has been observed by neutron diffusion [2] but recent studies using dielectric measurements have exhibited interesting properties that can be precisely characterized and open the way for a better understanding of interfacial water [3–5].

Contrary to the neutron investigations, these dielectric measurements are not probing the interfacial water attached to the surface of a protein in solution, but they study weakly hydrated protein powders, which are covered by a small amount of water (less than a monolayer). When the water coverage increases, the bound water molecules form clusters that exhibit a percolation transition detectable by a sharp increase of the protonic conductivity [6]. Moreover, recent studies of the temperature dependence of the dielectric relaxation exhibited a freezing of the proton dynamics associated to the divergence of the longest relaxation time [3,4] indicating that the hydrated protein is a *proton glass*. Above the transition temperature ( $\approx 270$  K), the relaxation is non-Arrhenius and well described by a Vogel-Fulcher law, while at low temperature an Arrhenius temperature dependence is detected. It is interesting to notice that similar results have been obtained for two-dimensional water in vermiculite clay [7], with a non-Arrhenius temperature dependence in the range 215–265 K.

Measurements of the proton mobility are closely linked to the dynamics of the water molecules themselves. Early studies of proton mobility in ice [8] had introduced the polaron concept suggesting that protons were not moving indepen-

dently of the surrounding water molecules. Recent quantum mechanical calculations [9,10] confirm that the dynamics of protons is governed by solvent fluctuations. These results suggest that, in order to analyze the dielectric measurements on proton transfer, one must first study the dynamics of the hydration-water molecules themselves.

In this work our aim is to propose and investigate a minimal model of protein hydration water. Using the full power of supercomputers, it is possible to proceed to molecular dynamics simulations of realistic models of water [1], and nowadays similar simulation of an hydrated protein is achievable although the very long time scales necessary to properly investigate a glass transition may be at the border of current possibilities. These studies are interesting but, even for elaborate models, their validity is nevertheless dependent upon the validity of the model and, for protein hydration water, the results would only be valid for the specific protein studied. Moreover, as realistic models are designed to describe all the properties of the water molecules as accurately as possible, such molecular dynamics simulations do not tell which of these properties is responsible of the observed behavior. One important feature of water molecules is their ability to form hydrogen bonds in specific directions. This suggests that *geometry* could be the crucial factor. To test this idea we have chosen a water model that emphasizes the directional character of the hydrogen bonds and is sufficiently simple to allow us to study various cases in order to draw conclusions that do not depend on the peculiarities of a given situation. We show that, if one takes into account the binding to the protein surface, the simple model does exhibit a glassy behavior. We analyze some properties of this transition and discuss its connection with the dielectric data.

### II. A MODEL FOR TWO-DIMENSIONAL WATER

Various models have been introduced to study different properties of water, but one of them appears to be particularly adapted for our purpose. This is the model proposed by Ben-Naim in 1971 [12] that has recently been revived in studies of the hydrophobic effect [13]. Water molecules can form hydrogen bonds in four directions in space, the two directions of the O—H bonds and two others determined by

electronic orbitals of the oxygen atom which form a tetrahedra with the O—H bonds. Those bonds determine the structure of ice and the local structure of liquid water and they are responsible for the anomalous properties of water such as its maximum density. The Ben-Naim model has been designed to describe the network of hydrogen bonds, but, since it was initially built for analytical studies, instead of the four ‘‘arms’’ in a tetrahedral geometry for a water molecule, it considers only three arms in a triangular arrangement in two dimensions. This simplification, which would have been a weakness for bulk water, is well adapted to describe hydration water because the molecules are hydrogen bonded to the surface by one of their four arms, leaving only the three others available for interactions with neighboring waters within a cluster. One interest of the Ben-Naim model is that it avoids the calculation of Ewald’s sum since the electrostatic interactions have been replaced by effective short range potentials. This is important for our purpose because the investigation of the glass transition requires studies on very long time scales.

In the Ben-Naim model, the interaction potential between two water molecules is a sum of two terms

$$U_{ij}(\vec{X}_i, \vec{X}_j) = U_{LJ}(R_{ij}) + U_{HB}(\vec{X}_i, \vec{X}_j), \quad (1)$$

where  $\vec{X}_i$  is a generalized three-component vector that specifies the coordinates  $x_i, y_i$  of molecule  $i$  in two dimensions and its orientation defined by the angle  $\alpha_i = (\vec{i}_1, \vec{x})$  between its first arm and the fixed  $x$  axis chosen as a reference,  $R_{ij}$  is the distance between molecules  $i$  and  $j$ ,  $U_{LJ}(R_{ij})$  is a Lennard-Jones potential between the centers of the molecules,  $U_{LJ}(R) = 4\epsilon[(\sigma_0/R)^{12} - (\sigma_0/R)^6]$ , parametrized by its energy scale  $\epsilon$  and range  $\sigma_0$ . The hydrogen-bond potential  $U_{HB}$  is split in two factors

$$U_{HB}(\vec{X}_i, \vec{X}_j) = \epsilon_H G_{\sigma'}(R_{ij} - R_H) \sum_{k,l=1}^3 G_{\sigma}(\vec{i}_k \cdot \vec{u}_{ij} - 1) G_{\sigma}(\vec{j}_l \cdot \vec{u}_{ij} - 1), \quad (2)$$

where function  $G_{\sigma}(x)$  is an unnormalized Gaussian function  $G_{\sigma} = \exp(-x^2/\sigma)$ . The parameter  $\epsilon_H$  is negative and sets the energy scale of the hydrogen-bond potential. The first factor indicates that an hydrogen bond has a preferred length  $R_H$ , while the second factor, which involves a summation over all pairs of indices  $k, l$  that label the three arms of molecules  $i$  and  $j$  respectively, is an angular term which indicates that hydrogen bonds are preferentially formed when an arm of molecule  $i$  is aligned with an arm of molecule  $j$ . The vectors  $\vec{i}_k, \vec{j}_l$  are unit vectors of the arms of the two molecules and  $\vec{u}_{ij} = \vec{R}_{ij}/R_{ij}$  is a unit vector along the direction that joins the center of the molecules. The angular term is chosen such that the strongest hydrogen bond is formed when two arms of the water molecules are perfectly collinear, and no distinction is made between donors and acceptors. It is this term that imposes the geometry of the network of hydrogen bonds. The

parameters  $\sigma'$  and  $\sigma$  determine the selectivity of the formation of hydrogen bonds with respect to the distance or orientation of the molecules.

The parameters of the model have been determined so that the interaction potential (1) gives an optimal fit of the potential of a standard model of water, the extended simple point charge (SPC/E) model [1], for various relative positions and orientations of the water molecules. They are  $\epsilon = 0.016$  eV,  $\sigma_0 = 2.625$  Å,  $\epsilon_H = -0.192$  eV,  $R_H = 2.5$  Å,  $\sigma' = 1.5715$  Å<sup>2</sup>,  $\sigma = 1.8359 \times 10^{-4}$ .

For free water molecules in two dimensions, the Ben-Naim model captures some experimentally observed properties of real water such as its density anomaly [12,13] and it freezes into an hexagonal icelike structure at low temperatures. In order to describe the properties of the hydration water, we must add an important component, the binding of the molecules to the surface that restricts their translational freedom. In our approach the surface is described by an underlying lattice that corresponds to the favorable sites for hydrogen-bond binding. The geometry of this lattice depends on the substrate (specific protein, glass, clay, for instance) but its general and essential feature is to be disordered but not fully random. Two water molecules cannot bound to two sites that are too close to each other, and, since we are interested in percolated water clusters, the binding sites must not be too far from each other either. In order to generate an underlying disordered lattice that meets these criteria, we start from a set of points randomly chosen inside a selected spatial region that determines the size of the cluster. We assume that these points are connected by Lennard-Jones potentials  $U_{LJ}(R)$  and we perform a small number of Monte Carlo (MC) steps that move the sites in order to reduce the energy of the lattice. The number of steps is not sufficient to bring the lattice close to its equilibrium structure (which would be a perfect triangular lattice) but sufficient to remove the major constraints introduced by points that are too close to each other. The amount of residual disorder can be varied by changing the number of MC steps, larger numbers leading to more regular lattices. A typical underlying lattice for a water cluster of 49 molecules is shown in Fig. 1.

Once an underlying lattice has been chosen, it is kept fixed for a set of numerical studies in which the temperature or the model parameters are varied. One water molecule is attached to each binding site by an harmonic potential  $U_{loc} = \epsilon_{loc} r^2$ , where  $r$  is the distance between the water molecule and its binding site in the underlying lattice. As the model is purely two dimensional, only the component of the distance that is parallel to the surface is considered. The value of  $\epsilon_{loc}$  depends on the substrate but it is not critical although it modifies quantitatively the results. We show here two cases that differ by one order of magnitude:  $\epsilon_{loc} = 0.244 \cdot 10^{-2}$  eV/Å<sup>2</sup> that is such that the frequency of the small amplitude vibrations of a water molecule in its translation parallel to the surface, that can be performed by bond rotations, is one-seventh of the frequency associated to the stretching of a hydrogen bond, and a larger value  $\epsilon_{loc} = 0.244 \cdot 10^{-1}$  eV/Å<sup>2</sup> corresponding to strongly bound water.

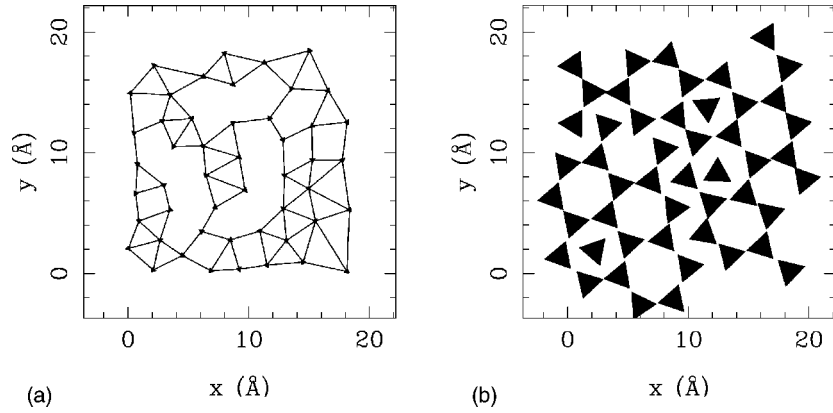


FIG. 1. (a) Example of underlying lattice of binding sites for a cluster of 49 water molecules. The points are the binding sites and lines have been drawn between each site and its three nearest neighbors to emphasize the topology of the lattice. (b) Structure of the water cluster obtained on this lattice at  $T=0.020$  eV. Each water molecule is shown as a triangle with its angles pointing in the direction of the three arms.

The presence of the underlying lattice is an important feature of the model because it has a structure and a connectivity that does not correspond to the equilibrium icelike structure that the water molecules would chose if they were freely moving in space. This introduces a *frustration* that strongly influences the dynamics of the water clusters.

### III. MONTE CARLO SIMULATIONS

The model of bound water has been investigated by MC simulations with a Metropolis algorithm. The energy includes the interaction between all the water molecules of a cluster and their binding energy to the underlying lattice. The MC simulations can be used for a dual purpose. Starting from an arbitrary configuration of the water molecules, the relaxation of the water cluster toward its equilibrium configuration determines its structure as a function of temperature. This is the usual application of the Monte Carlo method to investigate equilibrium properties. Once the energy is stabilized to its average equilibrium value, the MC calculation can probe the fluctuations in the water cluster with a fictitious time scale given by the number of MC steps. This provides an efficient alternative to molecular dynamics simulations at constant temperature, such as Langevin dynamics. Both methods use stochastic dynamics to explore the phase space but the MC calculations allows larger moves because it does not have to follow all the steps of an actual trajectory. There are of course drawbacks in this approach. First, although we henceforth call it “time” for brevity, the MC time scale does not correspond to the actual physical time, so that we cannot quantitatively connect the frequencies observed in the MC simulations to the dielectric measurements. Second, MC dynamics is only valid for the slow, and large amplitude motions, but cannot describe fast, small amplitude, motions such as phonon modes. Therefore molecular dynamics and Monte Carlo approaches are complementary. MC dynamics has, for instance, been successfully used to follow the slow dynamics of polymer melts for which a good agreement with experiments is found [14]. MC simulations are particularly suitable to study the glassy behavior of pro-

tein hydration water because they can efficiently investigate the rotational flips of the molecules. These motions involve hydrogen-bond breaking and reforming and are rare events at the scale of the vibrational motions of the molecules, especially in the vicinity of the glass transition, as shown by the low frequencies of the dielectric relaxation associated to the proton motions [3].

Structural data on the water cluster are provided by the static pair correlation function  $g(r)$  [Fig. 2(a)]. As one might expect, at high temperatures, the water molecules have large amplitude fluctuations around their binding positions and  $g(r)$  is similar to the pair distribution function of liquid water. It shows a peak around  $r=R_H$  and is almost featureless for large  $r$ . On the contrary, at low temperature,  $g(r)$  shows a series of peaks indicating a rather organized structure, which is however not as regular as in a solid because the binding of the water molecules to the protein surface prevents a crystallization into a regular ice structure.

The high and low temperature domains are separated by a smooth transition that can be detected by a broad maximum of the specific heat per particle [Fig. 2(b)], deduced from the fluctuations of the energy  $E$  of the cluster of  $N$  molecules in the MC iterations  $C_v = (\langle E^2 \rangle - \langle E \rangle^2) / [N(k_B T)^2]$ . The width of the transition region depends on the strength of the interaction with the underlying disordered lattice. Increasing  $\epsilon_{loc}$  broadens the transition.

In order to make the link with the dielectric measurements, it is necessary to characterize the fluctuations of the network of hydrogen bonds. This can be done by monitoring the *cage correlation function* [15]  $C(t)$  that measures the rate of change of the hydrogen bond network around each molecule. It can be defined as follows: at time  $t_0$ , determine for each molecule  $i$  the neighboring molecules and the indices of the arms of these molecules that are hydrogen bonded to the arms of molecule  $i$ . This defines the “cage” of molecule  $i$ . At a later time  $t$ , if nothing has changed in the cage of molecule  $i$ , set  $C_i=1$ , and, if anything has changed (index of a neighbor, broken hydrogen bond, or index of the linked arms), set  $C_i=0$ . The cage correlation function is then defined by the sum of the  $C_i$ 's over the cluster,  $C(t) = \sum C_i$ .

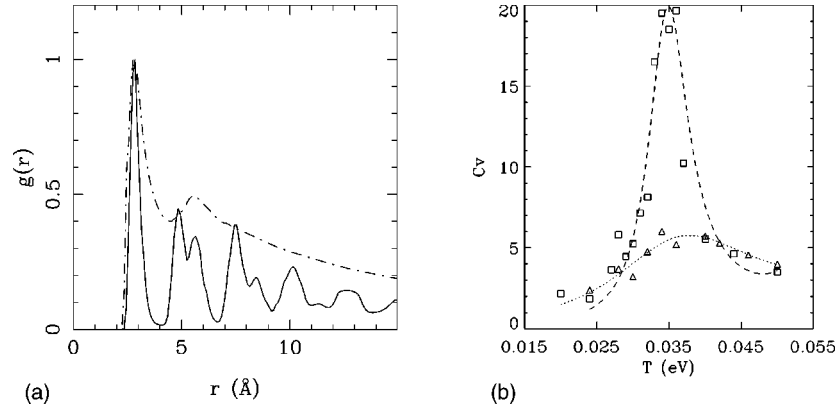


FIG. 2. (a) The pair distribution function  $g(r)$  at high and low temperatures. Full line:  $T=0.020$  eV, dash-dotted line:  $T=0.044$  eV. Notice that  $g(r)$  is normalized arbitrarily. It decays for large  $r$  because we investigate a finite cluster and not an infinite medium. (b) Specific heat per particle vs  $T$  (in energy scale) for a cluster of  $N=49$  water molecules. The points are the MC results: squares  $\epsilon_{loc}=0.244 \cdot 10^{-2}$  eV/Å<sup>2</sup>, triangles  $\epsilon_{loc}=0.244 \cdot 10^{-1}$  eV/Å<sup>2</sup>. The dash and dotted lines are guides to the eye obtained by fitting the numerical points with the expression  $C_v=A/(1+B(T-T_0)^2+C(T-T_0)^3)$ , where  $A$ ,  $B$ ,  $C$ , and  $T_0$  are parameters.

The time evolution of  $C(t)$  measures the persistence of a configuration of the network of hydrogen bonds. As  $C(t)$  only probes the breaking or formation of hydrogen bonds and not the small amplitude fluctuations of the water molecules, it is a quantity that can be calculated in a meaningful way by Monte Carlo dynamics.

At high temperature ( $T \geq 0.035$  eV), we observe a very fast decay of the cage correlation, which is not surprising since  $g(r)$  shows that the system is in a liquid state. The intermediate temperature range ( $0.025$  eV  $< T < 0.035$  eV) is the most interesting because the  $C(t)$  shows a slow, non-exponential decay, which is well described by a *stretched exponential* behavior

$$C(t) = C_0 \exp\left[-\left(\frac{t}{\tau}\right)^\beta\right] + C_1, \quad 0.3 \leq \beta \leq 0.6. \quad (3)$$

When  $T$  is decreased below  $0.025$  eV,  $\beta \rightarrow 1$ , i.e.,  $C(t)$  recovers a simple exponential decay, with a very long correlation time  $\tau$ , indicating that the water cluster is almost frozen.

As time evolves,  $C(t)$  decreases, but it does not drop to zero because the molecules are attached to the underlying lattice. The neighbors of each molecule are fixed so that the hydrogen bonds that a molecule can make belong to a finite set. Therefore, after an initial decay, which can be very slow

near the glass transition,  $C(t)$  settles to a constant average value but still fluctuates around this value because hydrogen bonds are continuously forming and breaking. The spectrum of its fluctuations, which can be calculated by the discrete Fourier transform of the recorded values of  $C(t)$ , provides another measurement of the dynamics of the formation and breaking of the hydrogen bonds [Fig. 3(b)]. It shows a power law  $C(f) \propto 1/f^\alpha$  over about three decades, with  $\alpha = 0.25 \pm 0.05$  in the transition region, indicating the existence of a broad range of time scales, dominated by slow fluctuations. This result is particularly interesting in the context of the latest dielectric measurements that have also exhibited an  $1/f^\alpha$  noise in the power spectrum of the dielectric fluctuations [5].

#### IV. DISCUSSION

The cage correlation function, which reflects the dynamics of the hydrogen bonds in the cluster, appears to be consistent with the dielectric measurements because, as discussed above, proton transfers are assisted by the dynamics of the water molecules. Therefore the stretched exponential relaxation of the cage correlation function, which is typical of a glassy behavior, can explain the experimental observation of a proton glass. The dynamics of the water cluster is

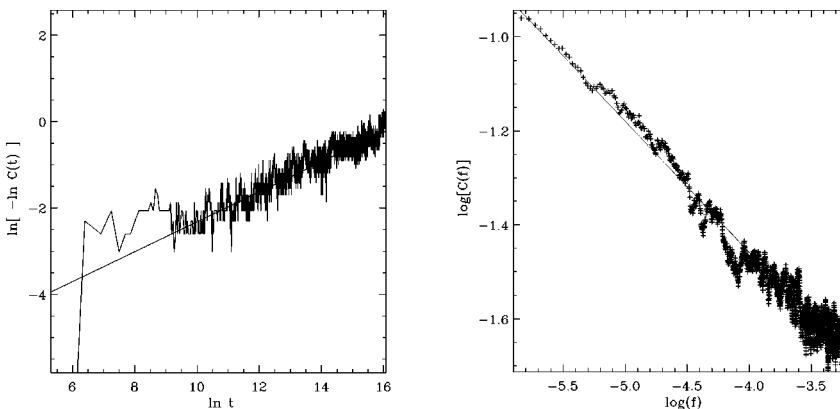


FIG. 3. (a) Time evolution of the cage correlation function  $C(t)$  at  $T=0.028$  eV. The figure shows  $\ln[-\ln C(t)]$  vs  $\ln(t)$ . (b) Spectrum of the fluctuations of the cage correlation function  $C(f)$ , in a decimal logarithmic scale, at  $T=0.032$  eV.



also consistent with the observation of an Arrhenius behavior of the dielectric measurements at temperatures below the glass transition. The very large increase of  $\tau$  that is observed in the MC dynamics at low  $T$  indicates that proton transfers assisted by water motions become extremely rare events. In the low temperature range, only jumps thermally activated over a high barrier can transfer protons from a molecule to the next. As the water clusters do not freeze in a regular ice structure but into a disordered lattice due to the binding to the underlying surface, the protons have to jump over a set of random barriers. For a Gaussian distribution of barriers, their diffusion coefficient can be shown to be  $D \propto \exp[-E^2/T^2]$  [16]. However, the small  $T$  range that has been investigated experimentally ( $250 \text{ K} < T < 270 \text{ K}$ ) is too restrictive to discriminate an Arrhenius kinetic law from a non-Arrhenius law such as  $\exp[-E^2/T^2]$ .

Therefore we have connected the glass transition observed in the proton dynamics to a glass transition of the water molecules themselves. The freezing of the water dynamics is consistent with the observation of a fast change in water thermal motions below 270 K for weakly hydrated myoglobin [11]. The Ben-Naim potential is only a simplified

description of water molecule interactions; however, it provides a good picture for the geometry of the hydrogen bonds. Combined with the frustration that comes from the underlying protein binding sites, it leads to a glassy behavior, showing that long range electrostatic interactions are not crucial. The present study cannot claim a complete description of “two-dimensional water,” but, as the MC studies of water clusters and dielectric measurements point toward the same direction, it suggests that the hydration water of proteins, and perhaps also of porous materials, could provide an interesting system to study some of the properties of supercooled or glassy water, and that dielectric measurements are a useful tool for such a study. The recent observation [17] of a similar smooth transition in the range 240–250 K by neutron scattering on water adsorbed on vycor glass suggests that the ideas included in the present model are sufficiently general to extend beyond the case of protein hydration water.

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