Relaxation Processes in Supercooled Confined Water and Implications for Protein Dynamics

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We show that the viscosity-related main (α) relaxation of confined water vanishes at a temperature where the volume required for the cooperative α relaxation becomes larger than the size of the geometrically confined water cluster. This occurs typically around 200 K, implying that above this temperature we observe a merged α-β relaxation, whereas below it only a local (β) relaxation remains. This also means that such confined supercooled water does not exhibit any true glass transition, in contrast to other liquids in similar confinements. Furthermore, it implies that deeply supercooled water in biological systems, such as membranes and proteins, generally shows only a local β relaxation, a finding of importance for low temperature properties of biological materials.

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The behavior of water in confined geometries and near solid surfaces is of central importance in nature, since most of the water in living organisms is closely associated with different kinds of biomolecules. The presence of this confined water is necessary for all living organisms [1–4], which makes it important to understand its properties. It is also of fundamental importance to understand how the structure and dynamics of the confined water affect its nearest surrounding, e.g., how the motion and function of proteins depend on the properties of its hydration water. In order to attack these important issues, we need a better understanding of structural relaxation processes in confined water, not only at ambient temperature but also in the supercooled regime, where, for instance, proteins begin to show anharmonic motions. It is at these low temperatures that it is most straightforward to gain insights into the important relation between protein and solvent dynamics. In order to study such supercooled water, we need to choose systems where the confinement is severe enough to avoid crystallization; i.e., the water clusters have to be smaller than the critical size of homogeneous nucleation [5].

The relaxation behavior of deeply supercooled liquids is generally described by the viscosity-related main (α) relaxation and one or several secondary (β) relaxation processes. The relaxation time τ_α of the α process generally shows some degree of non-Arrhenius temperature dependence, whereas the β processes tend to follow the Arrhenius law ($\log\tau \propto \frac{1}{T}$). Using Angell's fragility concept [6,7], a supercooled liquid is termed fragile when its viscosity or α relaxation time exhibit a highly non-Arrhenius temperature dependence, typical for ionic and van-der-Waals systems. In contrast, a supercooled liquid which shows a temperature dependence of the α relaxation time close to the Arrhenius law is denoted strong, reflecting that the material is held together by strong (commonly covalent) bonds forming a network structure. Recently, there has been experimental support [8–10] for the fact that supercooled highly confined water exhibits an apparent fragile-to-strong transition somewhere in the temperature range 180–250 K, depending on the size and geometry of the confinement as well as the strength of the interactions with the host material. In this Letter, we provide an explanation for this anomalous crossover and show that it is not due to a true fragile-to-strong transition but rather due to a vanishing of the strongly cooperative α relaxation. However, this does not mean that a real fragile-to-strong transition cannot occur for bulk water or bulklike water where the α relaxation is actually observed in the deeply supercooled regime.

In Fig. 1, we show temperature dependences of structural relaxation times obtained from dielectric spectroscopy and quasielastic neutron scattering (QENS). The normal temperature dependence of the α relaxation of a liquid is represented by both bulk propylene glycol (PG) as well as PG confined to a single molecular layer in the interplatelet space of a Na-vermiculite clay [11,12]. However, in the cases of water confined to two molecular layers in the same Na-vermiculite clay [8] and water confined in 10 Å pores of a molecular sieve [9], the behavior of the main relaxation time is completely different. The relaxation time is not only substantially altered compared to bulk water [13] (which evidently is not the case for the confined PG), it also shows an apparent fragile-to-strong transition, as discussed above. It should here be noted that an even more dramatic change of the temperature dependence of the relaxation time was observed at $T \approx 225$ K for water confined in the nanoporous silica MCM-41 [10]. Hence, these results are not unique but show the most common behavior for supercooled water in biological materials and other confinements. Indeed, as exemplified in Fig. 2 for ethylene glycol (EG), a "strong" (i.e., Arrhenius) behavior seems to be obtained for most deeply supercooled liquids provided that the confinement is severe enough. In the case of EG shown in Fig. 2, it is clearly seen that it is a rather fragile liquid in bulk. However, the cooperative α relaxation requires a certain number of molecules to be present, and, for the very severe confine-

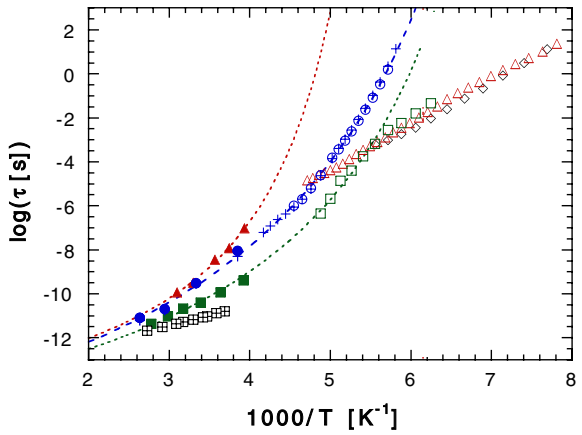

FIG. 1 (color online). Average relaxation times, obtained from dielectric spectroscopy (open symbols) and QENS experiments (solid symbols), for a 6 Å thick water layer in a fully hydrated Na-vermiculite clay (triangles) [8,24], for water in 10 Å pores of a fully hydrated molecular sieve (squares) [9,25], for water in hydrated hemoglobin (diamonds) [26], and for bulk water (squares with cross) [13]. For comparison, average relaxation times for bulk PG (crosses) and for PG confined to a single molecular layer in the Na-vermiculite clay (circles) [11,12] are shown to represent the normal liquid behavior. The relaxation times from QENS were obtained for $Q \approx 1$ Å^{-1}, since it has been empirically found [27] that QENS and dielectric data seem to agree for that Q value, although it should be noted that different types of motions are probed with the two techniques. In all cases, the dielectric data show the most pronounced relaxation process, which corresponds to the α relaxation if that is present and the β relaxation in the absence of the α process. This means that the high temperature data represent the merged α-β process, whereas the low temperature data correspond to the α relaxation in the case of bulk and confined PG and the more local β relaxation in the case of confined water. The temperature dependences of the α relaxation times are described (and extrapolated) by the Vogel-Fulcher Tammann (VFT) function $\tau = \tau_0 \exp[DT_0/(T - T_0)]$. Note also that the confinement of PG to a single molecular layer does not prevent the α relaxation to occur, and neither does it have any significant effect on the relaxation time.

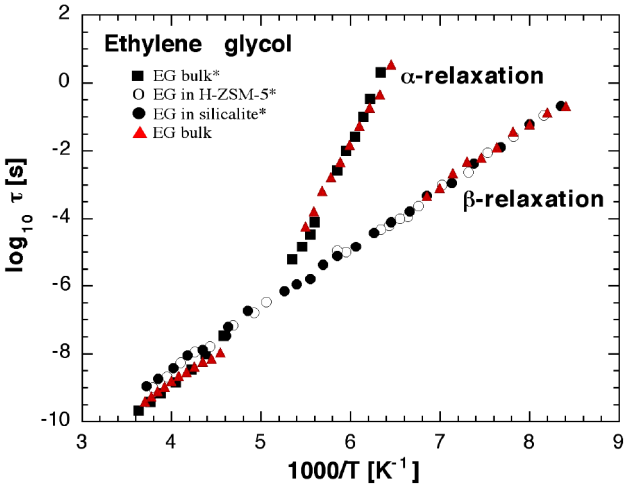

FIG. 2 (color online). Dielectric relaxation times of EG. Both the α and β relaxations are present for bulk EG, whereas only the local β relaxation can be observed for EG in the very severe confinements (pore diameter approximately 5.5 Å) of the zeolitic host materials silicalite (solid circles) and H-ZSM-5 (open circles). The data points, which in the legend are marked with an asterisk, are taken from Ref. [28], whereas triangles indicate our data. Note that the α relaxation of EG disappears in these severe confinements.

ments shown in Fig. 2, the space is small enough not to allow this number, which means that the α relaxation vanish and only the more local β relaxation remains. All density fluctuations are then relaxed through the local β relaxation. This gives rise to a similar apparent strong behavior in the deeply supercooled regime as observed for confined water in Fig. 1 and Ref. [10]. We will now give evidence that the apparent fragile-to-strong transition for supercooled confined water is due to an observation of a merged α-β relaxation at high temperatures and a pure β relaxation below the apparent transition.

(a) The temperature for which the relaxation time reaches 100 s is around 130 K, which means that, if this is the α relaxation time, a glass transition temperature T_g of the confined water is expected at about this temperature. However, the T_g of bulk water has recently been suggested to be >160 K [14,15], and, since the α relaxation at high temperatures is slower in confinement than in bulk (and the difference seems to increase with decreasing temperature), it seems unphysical to have a T_g around 130 K for the confined water.

(b) No calorimetric glass transition is observed, in contrast to other liquids in the same (e.g., the vermiculite clay) or similar confinements [16].

(c) The dielectrically observed process in the deeply supercooled regime shows all the typical features of a β relaxation, such as a symmetric peak shape in the frequency domain.

(d) In Ref. [10], the authors found a dramatic change of the nature of the dynamics from translational diffusion to local motions at the crossover temperature. This is the expected behavior when the α relaxation disappears and only the local β relaxation remains.

(e) In Ref. [17], a similar relaxation process was observed for rapidly quenched bulk water by electron spin resonance measurements, even in the so-called "no man's land" (150–235 K) where bulk water is mainly crystalline. This fact further supports that the observed relaxation process is of local character (in this case, the relaxation of water molecules in the interface between different crystalline regions).

(f) Although difficult, the α relaxation of deeply supercooled water can be observed if a delicate balance of the size of the water clusters is reached. The clusters have to be large enough (in all three dimensions) for the α relaxation

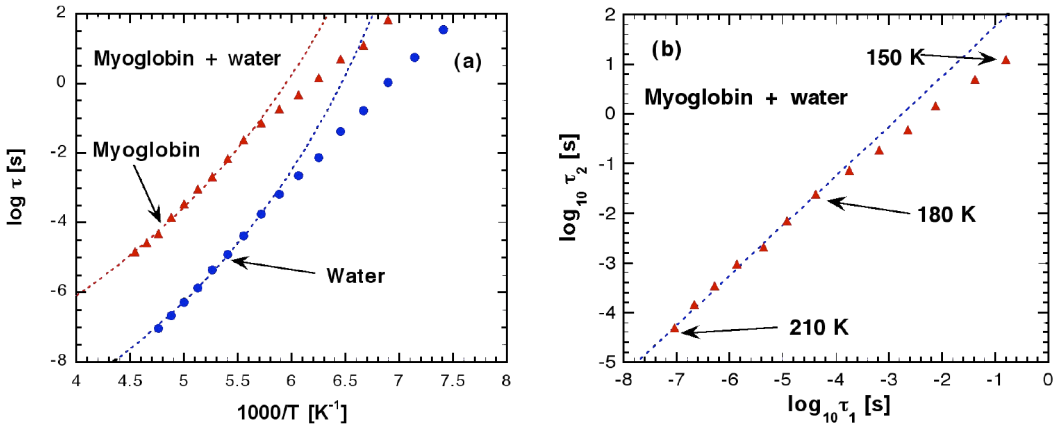

FIG. 3 (color online). (a) Temperature dependences of the relaxation times for the two fastest dielectric processes of myoglobin in water (0.8 g water per g protein). The fastest process (●) is due to interfacial water dynamics, whereas the slower one (▲) is due to protein motions. Both processes exhibit a crossover at about 180 K from a low temperature Arrhenius behavior to a high temperature non-Arrhenius (VFT) dependence, given by the dashed lines. (b) The logarithm of the relaxation times obtained for the slower (myoglobin) process is plotted as a function of the logarithm of the relaxation times for the faster (water) process. The dashed line is a guide to the eye and shows a slope = 1 (i.e., a linear dependence of the relaxation times with the faster process approximately 3 orders of magnitude faster than the slower one). Thus, above the crossover temperature there is a linear dependence between the relaxation times of the two processes, as expected for solvent slaved protein motions.

to appear while they are small enough to prevent crystallization. This has been achieved for water confined in bread [18], where the high temperature α-β relaxation splits into one fast (similar to the one shown in Fig. 1) and one slower relaxation process in the deeply supercooled regime. In addition, a calorimetric T_g is observed at about the same temperature (175 K) as the slower process reaches a relaxation time of 100 s, suggesting that this slower process corresponds to the α relaxation and the faster one to the β relaxation of the deeply supercooled water [18].

All these experimental findings strongly suggest that the common process of deeply supercooled confined water, shown in Fig. 1, is due to a β relaxation, which means that the α relaxation disappears in the same temperature region as the apparent fragile-to-strong transition occurs. Thus, the deeply supercooled confined water shows a similar behavior as the most severely confined liquids shown in Fig. 2, but the anomaly for water is that the vanishing of the α relaxation occurs already at comparably moderate confinements where other liquids show a strong α relaxation and glass transition. The reason for this is most likely that deeply supercooled water requires an exceptionally extended three-dimensional hydrogen bonded network [19] in order to show the α relaxation, in contrast to most other liquids, where only a few connected molecules (or lower dimensions) are enough for the α relaxation to appear. This also suggests that the α relaxation of deeply supercooled water is due to collective motions of a large number of water molecules.

Finally, we discuss the biological implications of the fact that hydration water around proteins and other biomolecules is lacking the viscosity-related α relaxation below the apparent transition temperature. Today, there is considerable evidence [20–23] that solvent motions are essential for the dynamics and functioning of proteins. This fact is further supported by Fig. 3, which shows that the fastest dielectrically observed protein (myoglobin) process exhibits the same temperature dependence as its solvent (water) dynamics at temperatures above 180 K. At such high temperatures, both processes show non-Arrhenius temperature dependences, as expected for cooperative processes as the α relaxation. However, at lower temperatures, where only local solvent motions occur, both relaxation times show similar Arrhenius temperature dependences (although the exact linear relation is now lost). This finding suggests that only local protein motions can occur when no α relaxation is present in the solvent. Therefore, the results support a recent study where it was shown [20] that only local motions in proteins are determined by the local β relaxation in the hydration shell, while the functionally most important protein motions are more global in character and are governed mainly by the viscosity-related α process in the solvent [21,22]. This implies that, in the deeply supercooled regime (in this case, below 180 K), where only the β relaxation of the noncrystalline water is present, the biologically most important protein motions cannot occur, in possible contrast to proteins in solvents that exhibit also the viscosity-related α relaxation (provided that the solvent does not crystallize in the given temperature range). Hence, other solvents than water are likely to be better suited to promote protein dynamics at these low temperatures.

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[1] *Biophysics of Water*, edited by F. Franks and S. Mathias (Wiley, London, 1983).
[2] K. Luby-Phelps, F. Lanni, and D. L. Taylor, Annu. Rev. Biophys. Biophys. Chem. **17**, 369 (1988).
[3] J. A. Rupley and G. Careri, in *Advances in Protein Chemistry*, Vol. 41 (Academic, San Diego, 1991), p. 37.
[4] S. B. Zimmerman and A. P. Minton, Annu. Rev. Biophys. Biomol. Struct. **22**, 27 (1993).
[5] B. Krämer *et al.*, J. Chem. Phys. **111**, 6521 (1999).
[6] C. A. Angell, J. Non-Cryst. Solids **131–133**, 13 (1991).
[7] C. A. Angell, Science **267**, 1924 (1995).
[8] J. Swenson, R. Bergman, and S. Longeville, J. Chem. Phys. **115**, 11 299 (2001).
[9] J. Swenson, H. Jansson, W. S. Howells, and S. Longeville, J. Chem. Phys. **122**, 084505 (2005).
[10] A. Faraone, L. Liu, C.-Y. Mou, C.-W. Yen, and S.-H. Chen, J. Chem. Phys. **121**, 10 843 (2004).
[11] R. Bergman, J. Mattsson, C. Svanberg, G. A. Schwartz, and J. Swenson, Europhys. Lett. **64**, 675 (2003).
[12] J. Swenson, G. A. Schwartz, R. Bergman, and W. S. Howells, Eur. Phys. J. E **12**, 179 (2003).
[13] C. Rønne, P. O. Åstrand, and S. R. Keiding, Phys. Rev. Lett. **82**, 2888 (1999).
[14] V. Velikov, S. Borick, and C. A. Angell, Science **294**, 2335 (2001).
[15] Y. Z. Yue and C. A. Angell, Nature (London) **427**, 717 (2004).
[16] S. Cerveny, J. Mattsson, J. Swenson, and R. Bergman, J. Phys. Chem. B **108**, 11 596 (2004).
[17] S. N. Bhat, A. Sharma, and S. V. Bhat, Phys. Rev. Lett. **95**, 235702 (2005).
[18] S. Cerveny, G. A. Schwartz, R. Bergman, and J. Swenson, Phys. Rev. Lett. **93**, 245702 (2004).
[19] H. E. Stanley and J. Teixeira, J. Chem. Phys. **73**, 3404 (1980).
[20] P. W. Fenimore, H. Frauenfelder, B. H. McMahon, and F. G. Parak, Proc. Natl. Acad. Sci. U.S.A. **101**, 14 408 (2004).
[21] P. W. Fenimore, H. Frauenfelder, B. H. McMahon, and F. G. Parak, Proc. Natl. Acad. Sci. U.S.A. **99**, 16 047 (2002).
[22] M. Tarek and D. J. Tobias, Phys. Rev. Lett. **88**, 138101 (2002).
[23] D. Vitkup, D. Ringe, G. A. Petsko, and M. Karplus, Nat. Struct. Biol. **7**, 34 (2000).
[24] R. Bergman and J. Swenson, Nature (London) **403**, 283 (2000).
[25] H. Jansson and J. Swenson, Eur. Phys. J. E **12**, S51 (2003).
[26] H. Jansson, R. Bergman, and J. Swenson, J. Phys. Chem. B **109**, 24 134 (2005).
[27] A. Arbe, A. Alegria, J. Colmenero, S. Hoffmann, L. Willner, and D. Richter, Macromolecules **32**, 7572 (1999).
[28] A. Huwe, F. Kremer, L. Hartmann, Th. Kratzmüller, H. G. Braun, J. Kärger, P. Behrens, W. Schwieger, G. Ihlein, Ö. Weiß, and F. Schüth, J. Phys. IV (France) **10**, 59 (2000).