Letter to the Editor

Reply to the comment by Graziano on "The hydrophobic effect and its role in cold denaturation"

Giuseppe Graziano comments [10] our article, in particular its interpretation of the hydrophobic effect [6] and cold denaturation [5]. In his comment Graziano states that "the fundamental point is that the pictorial 'iceberg' model is not supported by experimental evidence" and that there are "several questionable sentences that might lead to misunderstandings of the hydrophobic effect". The hydrophobic effect and its physical origin has been debated more or less continuously since late 1930s [19]. Some of the seminal papers are the theoretical works of Frank and Evans [8] which proposed the so-called "iceberg model", the 1959 paper by Kauzmann on denaturation [12], and the 1973 paper by Stillinger [18] which sets the foundation of the so-called Scaled Particle Theory. Our view, which is stated several times in the article, is that the picture provided by the iceberg model is useful but should not be taken literally. As such we find it unfortunate that the iceberg model is often used either as an all-explaining theory of hydrophobicity or as a failure relying on concepts from rigid ice structures. To our opinion Graziano seems to have missed the key points of our paper and his claims regarding our works and the hydrophobic effect in general are oversimplified and misleading. Thus, we wish to present a detailed analysis on the relevant issues in our Reply here. We proceed by first summarizing key experiments and after that discuss results from modeling and theory.

Graziano dismisses the iceberg model on grounds that no structural differences between solvation shell and bulk water have been observed in the recent neutron scattering experiments of Buchanan et al. [3]. However, these experiments should not be taken on absolute grounds since scattering data have to be interpreted using empirical simulations and not by direct observations. As such, another scenario has also been suggested (although with reservations) by the authors to account for their experiment: "There is also the possibility that the structure is enhanced near the methane (e.g., narrower, more pronounced peaks) but further away the water is more disordered (broader peaks) so that there is little net effect on the average structure determined here" [3]. This second scenario agrees with the molecular description of the iceberg model and is corroborated by X-ray absorption fine structure (EXAFS) spectroscopy experiments [2], which unambiguously show similarities between the hydrophobic hydration shell of a noble gas atom in the liquid state and that found in the solid clathrate. These are considered the first direct experimental evidence in favor of the iceberg model.

Perhaps the most notable evidence supporting the *qualitative* iceberg picture comes from the ultrafast non-linear femtosecond mid-IR spectroscopy experiments of Bakker et al. [15,1]. They show directly that there is a clear difference in the *dynamics* of water molecules in the solvation shell and in bulk. Thus, the solvation shell is stable, as the significant slowing down of mobility indicates and as has been proposed in the "iceberg" model of Frank and Evans. In particular, Bakker et al. report that the reorientation time is about four times slower in the solvation shell as compared to bulk (2.5 ps vs. >10 ps). These experiments are able to probe time scales faster than the exchange time between the solvation shell and bulk water thus having high enough temporal and spatial resolution. It should be noted that mid-IR spectroscopy data are not isolated in discriminating between the dynamics of shell and bulk water. Already 15 years ago Haselmeier et al. [11] were able to measure a clear difference in the rotational and translational motion of bulk and solvation water using ^{1}H spin–echo technique. More recently, Wachter et al. [21] made similar conclusions using dielectric relaxation spectroscopy.

Despite all the evidence in favor of the iceberg model, interpretation of experiments is not a trivial matter and in part explains the constant debate around the hydrophobic interaction. For example Qvist and Halle [14] point out, with respect to the mid-IR experiments, that "no existing experimental technique can directly measure the rate of water rotation in the hydration shell; a model-dependent interpretation step is always involved". On the modeling side, Laage et al. [13] used MD simulations to challenge the re-emerged iceberg view and they proposed that the re-orientational dynamics measured by Bakker et al. is better described by a jump model.

On the theoretical side water can make up to four directional hydrogen bonds that point towards the edge of a tetrahedron. As such it assumes structures similar to other tetrahedral materials such as, for example, silicon and germanium (which are covalently bonded). Based on our current understanding of covalent tetrahedral materials, it is inconsistent to claim that shell water is more disordered than bulk water—as done by Graziano. When a surface is created in covalent tetrahedral materials, surface reconstruction occurs in order to render the dangling hybrids of surface atoms more tangential to the surface. This occurs not only in the crystalline state [7] but also in the amorphous state and on small surfaces of the size of an atom, i.e., vacancy [20]. Directionality of the dangling hybrids (that account for covalent bonds) is the pre-requisite for this type of surface reconstruction and it is a property shared by hydrogen bonds in water. Thus, ordering of shell water is expected on the premises of hydrogen bonds only. What seems to be the relevant question to us is whether this increased ordering is enough to account for the hydrophobic interaction.

Probably the argument that most highlights the importance of hydrogen bonds is the fact that it is difficult to find in nature other liquids that show a strong attractive force imbalance similar to the one found between water and non-polar molecules [4]. This imbalance is due to hydrogen bonds and accounts for the hydrophobic effect. The small size of water should not be underestimated and might be responsible for the "universal" behavior of the hydrophobic effect: it affects non-polar molecules of all sizes and shapes.

Due to its small size, water can circumvent all types of non-polar molecules. Thus, what makes water so special as to be able to produce a universal hydrophobic effect is the ability of such a small molecule to make many hydrogen bonds.

As explained in our paper, Frank and Evans noted that without an ordered hydration shell, mixing non-polar solutes in water would be accompanied by a positive heat of mixing related to an increase in the number of broken hydrogen bonds in shell water. This is, however, not the case and as such the number of hydrogen bonds in shell water is at least equal to the number of hydrogen bonds in bulk water. Contrary to what Graziano claims it cannot thus always hold that $f_s > f_b$. In the literature, f_s and f_b have been computed from simulations. Using all-atom simulations of water Stillinger et al. [18] found a slight increase in the number of hydrogen bonds in the hydration shell at ambient conditions which supports the iceberg picture of Frank and Evans. Silverstein et al. [16] computed this quantity for different values of temperature (Fig. 4 of their paper). They found a crossover temperature below which $f_s < f_b$ and above which $f_s > f_b$. The former situation applies to ambient conditions and agrees with an increased ordering of the hydration shell molecules as expected from the iceberg model. In their insightful paper, Silverstein et al. discuss the implications of such a finding and argue that the parametrization used in Muller's model is not consistent with all-atom simulations and their own results. Therefore, they are not robust as claimed by Graziano.

At this point we should mention that although Graziano claims that the main cause of the hydrophobic effect is "solvent-excluded volume effect" he uses Muller's model to discuss the hydrophobic effect. As discussed in our paper the only ingredient of Muller's model is hydrogen bonding: "solvent-excluded volume" does not enter into the model. This approach seems highly inconsistent to us. Also, in a recent comment, Graziano writes [9]: "Water molecules reorganize around a non-polar spherical solute to avoid the loss of *H-bonds*. This mechanism is always operative (. . .)". Again this seems inconsistent with his comment to our paper where he mentions that 'the hydration shell is more disordered than bulk water".

It is important to point out that the main goal of our paper is to discuss the *temperature dependence* of the hydrophobic effect and show how this dependence leads to cold denaturation. As it has been pointed out by Southall and Dill [17], no attempt has been made by scaled particle theory to explain temperature dependence of the hydrophobic effect. This explains why this part of the literature has not been included in our paper and we will be happy to cite such achievements in our future publications as we become aware of them.

The point of the above discussion is to demonstrate that the field of hydrophobic effect is very much alive. At the moment, there is no single model that can explain all observations. As what comes to the iceberg model, it is a model that has been valuable both in providing insight and inspiring research in this exciting field. Unfortunately it tends to be taken too literally due to its name. As David Chandler pointed out in his review [4] a few years ago, hydrophobicity is a multifaceted phenomenon which we have started to understand only recently.

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