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Unusual dynamic properties of water near the ice-binding plane of hyperactive antifreeze protein

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The dynamical properties of solvation water of hyperactive antifreeze protein from *Choristoneura fumiferana* (*Cf* AFP) are analyzed and discussed in context of its antifreeze activity. The protein comprises of three well-defined planes and one of them binds to the surface of ice. The dynamical properties of solvation water around each of these planes were analyzed separately; the results are compared with the dynamical properties of solvation water of ice around its two crystallographic planes: *basal* and *prism*. Three main conclusions are inferred from our investigations. The first one is that the solvation shell of *Cf* AFP does not seem to be particularly far-ranged, at least not beyond what is usually observed for proteins that do not interact with ice. Therefore, it does not appear to us that the antifreeze activity is enhanced by a long-ranged retardation of water mobility. Also the correlation between the collective mobility of water and the collective mobility of protein atoms highly resembles the one measured for the protein that does not interact with ice. Our second conclusion is that the dynamical properties of solvation water of *Cf* AFP are non-uniform. The dynamics of solvation water of ice-binding plane is, in some respects, different from the dynamics of solvation water of the two remaining planes. The feature that distinguishes the dynamics of solvation water of the three planes is the activation energy of diffusion process. The third conclusion is that—from the three analyzed solvation shells of *Cf* AFP—the dynamical properties of solvation water of the ice-binding plane *resemble* the most the properties of solvation water of ice; note, however, that these properties *still clearly differ* from the dynamic properties of solvation water of ice. [http://dx.doi.org/10.1063/1.4931922]

INTRODUCTION

Organisms exposed to temperatures below 0 °C are capable of surviving in the cold environment thanks to many evolutionary adaptations. To a certain extent, they can protect themselves against injuries caused by ice formation, using proteins capable of interaction with the surface of ice. These are so-called ice-binding proteins (IBPs) and ice-nucleating proteins (INPs).[1,2] Among the ice-binding proteins, two groups called antifreeze proteins (AFPs) and antifreeze glycoproteins (AFGPs) are distinguished.[1] They help to keep the bodily fluids in a supercooled state. The antifreeze agents that display exceptionally high antifreeze activity are called hyperactive. The protein investigated here is one of them.

Many theories are used to explain the mechanism of action of AFPs, as it has been sketched in our previous paper.[3] The most popular one states that AFPs act by adsorption to the ice surface, and therefore, they restrict the growth of the ice front to regions between the adsorbed protein molecules. Because of that, any subsequent growth of the ice crystal occurs on a curved interface, and as a result, it becomes less thermodynamically favorable[4] due to the so-called Gibbs-Thomson effect.

As mentioned by Sharp,[5] to adsorb effectively, AFPs must be able to "notice" one phase of water, ice, in a great excess of another phase, liquid. Various AFPs are even able to distinguish different crystallographic planes of ice and to adsorb to only one or two of them.[6–9] The ability to bind to two ice planes, and especially to the basal one, has been proposed to be a key prerequisite for an AFP to be hyperactive.[6] In the process of binding of AFPs to ice, both hydrophobic and hydrophilic interactions appear to play a significant role.[10,11]

To adsorb to the ice surface, an AFP must diffuse through the solvation layer of ice, which is quite thick, reaching about 1-2 nm.[12–14] It has been suggested that the solvation water between ice and an AFP freezes when the protein is sufficiently close.[15] Taking this into account, it is hardly possible to perceive the mechanism of binding of AFPs to ice as a simple protein-ligand recognition. Therefore, the role of water enclosed within solvation layer around both surfaces (the one of the protein and the one of the crystal of ice) should not be omitted when discussing the mechanism of action of AFPs.

In our previous paper,[3] we have reported unusual structural properties of solvation water around the ice-binding surface of hyperactive antifreeze protein from *Choristoneura fumiferana* (*Cf* AFP). Now we wish to continue this project by taking into consideration the dynamic properties of solvation water around the same AFP.

There is no consensus regarding the mobility of solvation water of antifreeze agents and the significance of the dynamics. Recently, Ebbinghaus and coworkers[16–19] used increasingly popular terahertz spectroscopy to detect a long-ranged retardation of water dynamics in the solvation shells of AFPs and

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AFGPs.[17] They considered this phenomenon to be a possible factor disfavoring the freezing of water. The same authors also investigated an antifreeze peptide and its mutants[19] and found that the most active peptides had extended, long-range (up to 2 nm) dynamical hydration shells. According to them, this shell may be particularly important for enhancing the antifreeze activity of proteins at their low concentrations. On the other hand, the interpretation of these results has been questioned by Halle,[20] who convincingly argued that these conclusions are an interpretational artifact. Also a recent paper[21] clearly indicate that dynamic properties of water around antifreeze proteins do not differ that significantly from the ones around typical proteins. Generally, it seems that the dynamic properties of solvation water of various proteins are not drastically different from each other even at deeply supercooled solutions.[22] Likewise, the conclusions reached by Modig *et al.*[23] were different from the conclusions presented by Ebbinghaus *et al.* These authors conducted the experimental study of the dynamics of solvation water of *Tm*AFP and concluded that the dynamics is qualitatively comparable to typical dynamics of solvation water of proteins that do not interact with ice. The changes in water mobility were neither particularly far-ranged nor the dynamics was particularly retarded, with the exception of several ordered water molecules on the ice-binding plane. Moreover, the aforementioned considerations are not consistent with the conclusions reached by Nutt and Smith,[15] who investigated *Cf* AFP solvation water by computer simulations. These authors observed increased mobility of the solvent at some distance from the two protein surfaces which do not interact with ice. It was proposed that this phenomenon helps keep the adjacent water in a liquid state. However, the conclusions regarding the accelerated dynamics were partially questioned in our previous paper,[3] where we used the same water model as the one used in Ref. 15 (TIP5P).

METHODS

Simulation procedure

The results were obtained using computer simulations, with the molecular dynamics package Amber10,[24] and ff03 force field, suitable for proteins.[25]

Systems setup

Two different types of systems had to be constructed, as follows:

1. systems consisting of *Cf* AFP molecule immersed in SPC/E water,
2. systems consisting of ice cuboid immersed in liquid SPC/E water.

Cf AFP in water

The initial coordinates of *Cf* AFP molecule were taken from Protein Data Bank (PDB ID: 1L0S). The original file contained one iodated tyrosine (not present in the natural protein), which was changed into tyrosine. Moreover, two counterions (chloride anions, Cl^-), and all missing residues and atoms were added in LEaP program, which is a part of Amber package. Amino acids with charged side chains were Arg, Asp, Glu, and Lys. Finally, the protein was placed inside a truncated octahedral box and solvated with SPC/E water, with a minimal distance between the protein and the box walls equal to 2.5 nm. As a result, each system contained approximately 15 000 water molecules. A preliminary period of equilibration in *NPT* conditions lasted about 2.0 ns. The appropriate temperature (from 240 to 300 K with a 10° interval between isotherms) was kept constant by the weak coupling to an external bath (τ_T = 1.0 ps) using Berendsen thermostat.[26] The pressure (1 bar) was kept constant by the weak coupling[26] method (τ_p = 1.0 ps). The particle-mesh Ewald method was used for electrostatic interactions, and the lengths of chemical bonds involving hydrogen atoms were fixed using SHAKE. A cutoff of 1.2 nm for nonbonding interactions was used.

The equilibrated system was simulated in *NpT* conditions, using time step equal to 1 fs for calculations of the velocity auto-correlation functions and in *NVE* conditions, using time step equal to 2 fs (calculation of correlations between the mobility of water molecules and the atoms of the protein). Trajectories were saved every single step (i.e., 1 fs for *NpT* and 2 fs for *NVE*). Total simulation time for production run was equal to 8–10 ns at each temperature. More details about the simulation procedure are included in the supplementary material.[27]

Ice cuboid in liquid water

We built a cubic crystal of ice that consisted of 2090 SPC/E water molecules as described previously.[3] The crystal was solvated with water up to 2.5 nm from its surface and placed in a rectangular box. In the starting configuration of ice, the orientation of bonds between oxygen and hydrogen atoms was ordered. In the real ice Ih, these bonds are distributed randomly. To facilitate and speed up the process of reorientation of water molecules, the system was heated up to 1000 K in *NVT* conditions. To avoid the crystal's destruction, its oxygen atoms were restrained using a harmonic potential (force constant 100 kcal · mol^{-1} A^{-2}). Subsequently, one configuration with fairly low overall dipole moment of ice cube was chosen. It was a starting point for further equilibration in *NPT* conditions at the same temperature as above (240 ÷ 300 K) and using restraining potential for oxygen atoms of ice (a force constant was equal to 10 kcal · mol^{-1} A^{-2}). Next, the proper production run begun. Total simulation time was equal to 8 ns, the time step of simulation was equal to 2 fs, and trajectory (for calculations of the velocity auto-correlation functions) was saved after each step. Two opposite faces of the ice cuboid were basal planes, while the two other were primary prism planes.

Definition of solvation layers

In our previous paper,[3] we used a thick solvation shell, reaching up to 1.2 nm from the backbone of the protein. To ensure consistency between the structural results obtained previously and the dynamic ones, we now used the same definition of solvation layer for calculations of diffusion

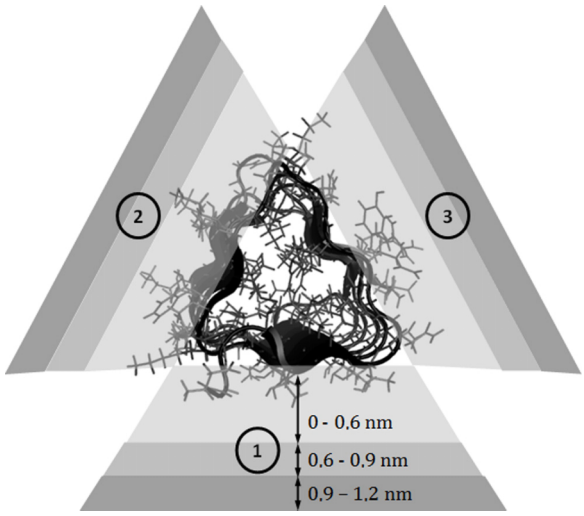

FIG. 1. The definition of solvation shells of the three planes of the *Cf* AFP molecule. Plane 1 is the ice-binding plane.

coefficients. Additionally, we divided the thick layer into three sub-layers, ranged from 0 to 0.6, from 0.6 to 0.9, and from 0.9 to 1.2 nm—see Figure 1 for illustration—and the properties of water present within subsequent solvation layers of *Cf* AFP were investigated.

We are aware that our definition of solvation layer may seem unconventional. Therefore, additionally, we used a commonly accepted definition based on the distance between water molecules and the protein surface. Diffusion coefficients of solvation water of the protein were calculated in *NVE* conditions for three selected temperatures: 240 K, 270 K, and 300 K. These results are included in the supplementary material.[27]

Definition of parameters describing mobility of water molecules

The traditional and presumably the most rational measure of mobility of water molecules are diffusion coefficients. Denote the vector of translational velocity ($K = T$) or rotational velocity ($K = R$) of water molecule by $\boldsymbol{v}_K(t)$. The diffusion coefficients: translational (D_T), and rotational (D_R), may be calculated from the appropriate velocity auto-correlation function[28] using the Green-Kubo relation[29]

$$D_K = \frac{1}{3}\lim_{t\to\infty}\left(\int_0^t C_K(t)dt\right) \cong \frac{1}{3}\int_0^{T_c} C_K(t)dt. \qquad (1)$$

In this relation, T_c is the cut-off time, while $C_K(t)$ denotes the averaged (over all N_w water molecules included within solvation layer and over total number of analyzed frames) velocity auto-correlation function

$$C_K(t) = \langle \mathbf{v}_K(t_0)\cdot\mathbf{v}_K(t_0+t)\rangle, \quad 0 \le t \le T_c, \qquad (2)$$

where $\mathbf{v}_K(\tau)$ symbolizes translational ($K = T$) or angular ($K = R$) velocity vector of water molecule.

The cut-off time T_c should be long enough to ensure the convergence of the value of integral (1). It is usually achievable in picosecond time scale and this time does not exceed $10 \div 20$ ps. This time is temperature-dependent and increases with decreasing temperature. The monitoring of the position of a molecule within a relatively long time period ensures a complete convergence of the velocity correlation function to zero but is also troublesome in one regard. The molecule may travel between different solvation layers; as a result, it cannot be unambiguously ascribed to one of the layers. In our calculations, we used the cut-off time, T_c (which substitutes "infinity" in Eqs. (1) and (2)), equal to 1.5 ps for both the translational and rotational velocity autocorrelation functions. It ensures that average displacement of water molecule is small—even at 300 K it should not exceed 0.20 nm. Thus, according to our intentions, the value of the above integral reflects (average) dynamic properties of water within selected solvation layer,[28] and it is the main advantage of this approach.

However, the adopted procedure has also one defect: we are fully aware[27] that it leads to overestimation of the calculated D_T and D_R values obtained from Eq. (1)—this is the effect of neglecting the long-time tail in auto-correlation function, and this error is temperature-dependent. Fortunately, it does not seem very prominent. As it is discussed in the supplementary material,[27] it does not exceed 10% for the translation of pure SPC/E water. For rotation, the error seems to be virtually negligible. Anyhow, for the sake of precision, we had decided not to call the calculated values the diffusion coefficients, because strictly speaking they are not exactly equal. To avoid any misunderstanding, we used the term "parameter Δ_K" instead. Therefore, the equation to obtain it was

$$\Delta_K = \frac{1}{3}\int_0^{T_c} C_K(t)dt, \quad T_c = 1.5 \text{ ps}. \qquad (3)$$

Note that the procedure is the same for all of the solvation layers. Therefore, the observed differences in water dynamics between three investigated planes reflect physical reality and cannot be treated as a computational artefact. More explanations about the used procedure are included in the supplementary material.[27]

Collective dynamics of water and cross-correlation of protein-water collective motions

Recently, Heyden and Tobias[30] used computer simulations to find the correlation between motions of single surface atoms of the protein and motions of water molecules, located near fixed space point at a selected distance from the surface. The correlation reported by Heyden and Tobias was explained by the wave propagation, which engages water molecules and the surface atoms of a protein. The equation employed by Heyden and Tobias in their study used a Gaussian distribution to ascribe some velocity of neighboring water molecules to each space point $\mathbf{r}$. The width of the distribution was controlled by the value of σ. The equation is given below:

$$\boldsymbol{\rho}(t,\mathbf{r}) = \frac{1}{(2\pi\sigma^2)^{3/2}}\sum_i \mathbf{v}_i(t)\,\exp\left(\frac{-r^2}{2\sigma^2}\right), \qquad (4)$$

and $r = |\mathbf{r}_i(t) - \mathbf{r}|$. With this equation, we can obtain the vector of collective velocity, $\boldsymbol{\rho}$, of selected group of protein atoms or water molecules, present nearby a given space point, $\mathbf{r}$, at time

t. The position of ith element of the group (i.e., a protein surface atom or a water molecule) is denoted by $\mathbf{r}_i(t)$, while $\mathbf{v}_i(t)$ symbolizes the velocity vector of this element. Heyden and Tobias used σ equal to 0.4 Å. Their distribution was very narrow, with the closest molecule contributing most to the calculated value of $\boldsymbol{\rho}(t,\mathbf{r})$. We were interested in using a broader distribution to capture collective dynamics of water molecules over a greater space range. This is why we used σ equal to 0.4 nm. As it has been presented previously,[31] this equation can be used not only to calculate the collective velocity of a group of water molecules but also the collective velocity of a group of surface protein atoms. The correlations between motions of these groups of atoms were clearly visible with σ equal to 0.4 nm. This value ensures also that the diameter of the analyzed area on the protein surface is still small comparing to the diameter of the whole molecule.

We have decided to apply this concept to the case of *Cf*AFP molecule. The goal was to check whether the correlation between collective motion of protein atoms and collective motion of water molecules holds also for *Cf*AFP solvation shell and if it changes in comparison with a protein that does not display any antifreeze activity. The correlation was calculated as a function of distance from the protein surface, r. The time cross-correlation function between the collective velocities of protein atoms and water molecules is defined as[32]

$$C(r,t) = \frac{\left\langle \boldsymbol{\rho}_P(t_0,\mathbf{r}_P) \cdot \boldsymbol{\rho}_W(t_0+t,\mathbf{r}_W) \right\rangle}{\left\langle \left|\boldsymbol{\rho}_P(t_0,\mathbf{r}_P)\right| \right\rangle \left\langle \left|\boldsymbol{\rho}_W(t_0,\mathbf{r}_W)\right| \right\rangle}, \tag{5}$$

where $r = |\mathbf{r}_W - \mathbf{r}_P|$, $\boldsymbol{\rho}$ symbolizes vector of collective velocity, while indexes P and W describe protein and water, respectively. After expanding this function in a Fourier series, we obtain

$$C(r,t) = \sum_n c(r,\omega_n) \cdot \cos(\omega_n t - \varphi_n), \tag{6}$$

where $\omega_n = 2\pi n/T_0 = 2\pi\nu_n$. In our calculations, we used $T_0 = 4$ ps. The functions $c = c(r,\omega_n)$ and $\varphi = \varphi(r,\omega_n)$ represent, at fixed r, the amplitude spectrum and the phase spectrum of the function $C(r,t)$, respectively. Both of these spectra are the real functions of variable ω and they describe the amplitude density and the phase density of elemental harmonic components of the $C(r,t)$ function. The obtained amplitude spectra (for $\sigma = 0.4$ nm) are presented in Figure 3.

RESULTS

The analyzed protein is a prism-shaped AFP with one threonine-rich plane that adsorbs to the surface of ice (plane 1 in Figure 1) and two planes that remain in contact with the liquid water (planes 2 and 3 in Figure 1). The main conclusion drawn from our previous results[3] was that the structural properties of solvation water of the plane of the protein that adsorb to the ice surface are different from the structural properties of solvation water of the other two planes of the protein. Some of the structural characteristics of solvation water of the ice-binding plane and their temperature dependence resembled the properties of solvation water of ice. For this reason, we had decided to analyze the dynamical properties of solvation water of the three planes of the *Cf*AFP along with the properties of solvation water of ice. The purpose was to check whether the dynamical features would follow the pattern of the structural properties. The definition of the solvation shells can be found in Figure 1.

The presentation of our results is divided into two parts. In the first part, the temperature dependence (from 240 K to 300 K) of Δ_T and Δ_R parameters, determined for solvation water of the *Cf*AFP molecule, is described and compared with solvation water of ice. In the second part, we describe the collective dynamics of water, employing the method developed by Heyden and Tobias.[30] The results allow us to comment on the conclusions of Ebbinghaus *et al.*[16,19]

The local values of Δ_T and Δ_R parameters for water enclosed within solvation layer of *Cf*AFP

The temperature dependences of Δ_T and Δ_R parameters for solvation water from the first solvation shell of *Cf*AFP and ice are depicted in Figure 2. Two crystallographic planes of ice, basal and primary prisms, were also analyzed separately (these are the ones that the *Cf*AFP is said to adsorb to[4]). All of the results (for water within three solvation layers of the protein and ice crystal) are graphically presented in Figure S1 in the supplementary material.[27]

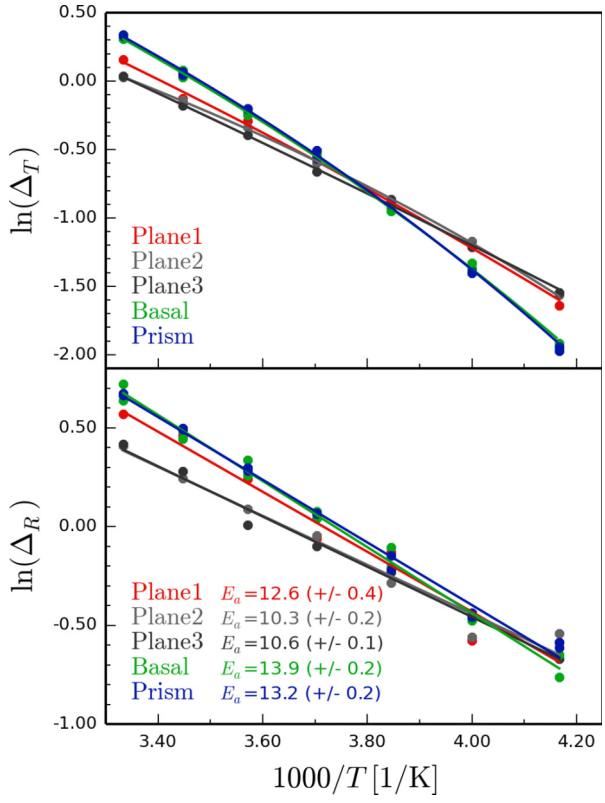

FIG. 2. Inverse temperature dependence of logarithms from translational (Δ_T, in units 10^{-9} m^2/s) and rotational (Δ_R, in units 10^{11} rad^2/s) parameters. Red and grey points represent the values for first solvation shells of the three planes of *Cf*AFP molecule. The blue and green points represent the values for solvation shell of basal and primary prism surfaces of ice. The points $(\ln(\Delta_T), 1000/\mathrm{T})$ are approximated with Vogel-Fulcher-Tammann equation. To the points $(\ln(\Delta_R), 1000/\mathrm{T})$, linear regression lines are added. The values of the slopes, E_a, presented in $\ln(\Delta_R) = f(1/T)$ plot are given in kJ/mol.

As it was stated in the section on Methods, we are aware that the definition of solvation layers used in this paper is uncommon. However, it did not affect any of our conclusions. To demonstrate it, we partly repeated our calculations, using a more wide-spread definition, based on the distance between water molecules and the protein surface. Three temperatures were selected: 240 K, 270 K, and 300 K. Results of these calculations are presented in Figure S2 (see supplementary material[27]).

The local values of Δ_K parameters indicate that the dynamical solvation shell reaches up to (0.9–1.2) nm from the backbone of the protein, although at the end of this distance (i.e., at 1.2 nm) the water dynamics is already almost bulk-like. As it was mentioned in the Introduction, several studies pointed out that antifreeze agents are surrounded by an extraordinarily long-ranged dynamical solvation shell, which reaches up to 2 or even 3.5 nm from the surface of the solute;[16,17] these authors also suppose that it may be important for the antifreeze activity. The values of mobility parameters obtained by us do not support the statement that the extent of the dynamical solvation shell is greater for *Cf* AFP than for proteins that do not interact with ice, which are said to have a dynamical hydration shell about 1-nm-thick.[33] Already at the distance from the protein surface from 0.7 to 1.0 nm (Figure S2[27]), their mean values are nearly bulk-like for solvation water of all three planes. The extent of the solvation shell appears to be a little greater when correlations of collective dynamics of water and dynamics of the protein atoms are taken into consideration, as will be discussed later.

A more singular feature of *Cf* AFP hydration is the temperature dependence of the investigated quantities. It is clear that the solvation water of the three planes responds differently to the changes of the temperature. The differences between the temperature dependence of Δ_T and Δ_R parameters are, understandably, best visible for water from the first solvation shell. The slope of the dependence and the values of the parameters are similar for planes not interacting with ice. The ice-binding plane differs from the two of them in these regards. When it comes to the solvation water of ice, the slope and the values of Δ_T and Δ_R parameters resemble the most the solvation water of the ice-binding plane (although the difference between them is still significant). This is especially visible for the Δ_R parameters.

As it is explained in the section on Methods, the Δ_T and Δ_R parameters may be greater than the actual diffusion coefficients D_T and D_R, because integral (1) is calculated only to 1.5 ps. In spite of that, we can be sure that including the long-time tail of velocity autocorrelation function would not cancel out the differences in the slopes of the functions $\ln \Delta_K = f(1/T)$ between the solvation layers of the three planes. The values of errors $(D_K - \Delta_K)$ are, presumably, the same for the three planes at given temperature. The errors increase in lower temperatures; therefore, the difference in the slopes of the functions $\ln D_K = f(1/T)$ may in fact be expected to be even more pronounced than the one found by us for the approximated parameters Δ_K. Therefore, we believe that the difference in the slopes of the functions $\ln \Delta_K = f(1/T)$ reflects the difference between the activation energy of translation and rotation. This energy appears to be slightly higher for solvation water of plane 1. This finding agrees with conclusion expressed by Duboue-Dijon and Laage:[21] the authors found greater temperature changes of the water reorientational dynamics near the ice-binding plane, comparing to the rest of the *Cf* AFP molecule surface. The slowdown increased monotonically with decreasing temperature. A slightly higher value of activation energy is also in accordance with our previous[3] results, which concerned structural properties of solvation water, because the activation energy can change as a result of structural changes. Different arrangement of surrounding atoms results in local differences in potential energy of intermolecular interactions. It changes the height of a barrier of rotation of a molecule. This way, our previous conclusions[3] concerning the unusual structure of solvation water can be related to the dynamical properties of water. The dynamics, as well as the structure, of solvation water of the ice-binding plane is different from the rest of the solvation water of the protein.

Long-range collective dynamics of solvation water around *Cf* AFP

As it was mentioned in the Introduction, recently Meister *et al.*[16] used far-IR spectroscopy (2.3 THz) to find that the dynamics of solvation water is altered up to even 2.0 nm. According to them, the modification of the dynamics, especially at long distances, is related to the activity of antifreeze agents.[16,17,19] However, the interpretation of their results seems to be controversial: Halle[20] argued that the data analysis conducted in the cited Refs. 16, 17, and 19 is incorrect, and the conclusions are an interpretational artifact.

The molecular dynamics simulation may be engaged to elucidate the problem because it could allow one to directly observe a hypothesized perturbation of collective water dynamics at long distances. Heyden and Tobias[30] attributed the absorption of the IR radiation to the collective dynamics of the water molecules. Thus, the analysis of the collective dynamics of water may help us find a relationship between the dynamics of solvation water and the antifreeze activity, suggested by Ebbinghaus *et al.*[16,19] It might be expected that the perturbation of collective dynamics of solvation water of *Cf* AFP will be more pronounced and will span greater distance than the dynamics of solvation water of a protein that does not display any antifreeze activity. This hypothesis was tested by us and the conclusions are below.

The results of the calculations of the correlations of collective dynamics of solvation water and the dynamics of the protein atoms are presented in Figure 3 and compared to the previously discussed[31] results of collective dynamics of solvation water of a tubulin dimer. It is apparent that there is no qualitative difference between the collective dynamics of solvation water of the ice-binding plane and the two remaining planes, as well as the solvation water of the tubulin dimer. There is a small difference in the magnitude and the scope of the correlation between the *Cf* AFP and the tubulin dimer. The correlation is detectable up to about 1.4 nm for *Cf* AFP and seems to vanish a little bit earlier for the tubulin dimer. Also, the values for near-zero frequency are higher for *Cf* AFP molecule. The explanation of the slightly higher correlation may be in the difference between diffusion

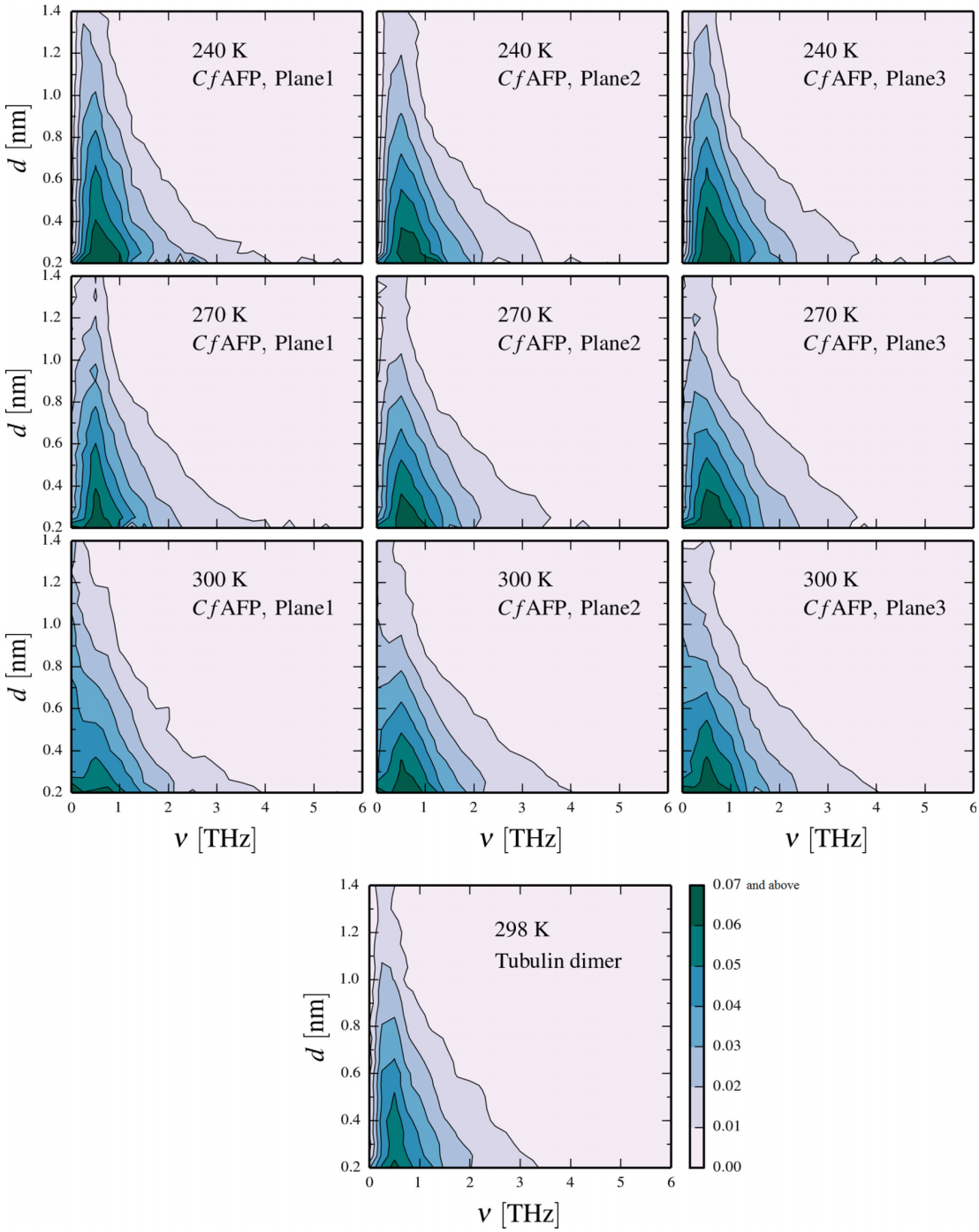

FIG. 3. Amplitude spectra of the velocity correlation function of the surface protein atoms (the three planes of *Cf*AFP and the tubulin dimer) and solvation water calculated according to Equations (5) and (6), using $\sigma = 0.4$ nm. The distance between water and protein is denoted by d, and the frequency is denoted by ν.

coefficients of these proteins. The *Cf*AFP molecule is smaller than the tubulin dimer. Therefore, its diffusion coefficient is greater. The rough estimate of the ratio of the diffusion coefficients (according to the Stokes-Einstein equation) is $D_{Cf\mathrm{AFP}}/D_{tubulin} = \sqrt[3]{(M_{tubulin})/(M_{Cf\mathrm{AFP}})} \approx 2.2$. The correlation of motions of protein atoms and water molecules can be partially attributed to the translation of the whole protein along with its solvation shell. Greater diffusion coefficients result in higher values of the amplitude spectrum for low frequencies. Therefore, in our opinion these results do not provide any evidence for a connection between the collective dynamics and antifreeze activity. Although the above conclusion is in conflict with the results presented by Meister *et al.*[16] and Ebbinghaus *et al.*,[16,19] this discrepancy seems to be understandable, taking into account the argumentation presented by Halle.[20]

CONCLUSIONS

The picture of the dynamics of solvation water of antifreeze agents emerging from the literature is blurred. The conclusions are often inconsistent and sometimes even mutually exclusive. We found some characteristics of solvation

water dynamics that are in agreement with our previous studies[3] and some literature data.

Main conclusions deduced from our results may be shortly summarized as follows. First of all, the solvation shell of *Cf* AFP does not seem to be particularly far-ranged, at least not beyond what is usually observed for proteins that do not interact with ice. It does not appear to us that long-ranged retardation of water mobility is a factor that enhances the antifreeze activity, as was suggested in several studies mentioned above. Also the correlation between the collective mobility of water and the collective mobility of protein atoms highly resembles the one measured for the protein that does not interact with ice.

At the lowest temperatures, the translational motion is slowed down the most for solvation water of plane 1. This observation is in accordance with one of the proposed mechanisms of action of AFPs which states that the solvation water between the protein and the ice surface may freeze upon the association.[15]

From the three analyzed solvation shells of *Cf* AFP, the dynamical properties of solvation water of the ice-binding plane were found to resemble the solvation water of ice the most. It should be noted, however, that these properties still clearly differ from the dynamic properties of solvation water of ice. Analogical conclusions were drawn by us previously[3] for structural properties. This statement applies, above all, to the temperature dependence of rotational diffusion coefficients. It is the feature that distinguishes the dynamical properties of solvation water of ice-binding plane from the two remaining planes the most. The higher energy barrier for rotation for plane 1 can be attributed to local structural changes of water.

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