Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Molecular dynamics study on the role of solvation water in the adsorption of hyperactive AFP to the ice surface.

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1. The histograms of the distances of the *Cf*AFP molecules from the ice surface.

On average, during the simulations the distances of the ice-binding surfaces of the *Cf*AFP molecule from the ice block plane (*basal* or *primary prism*) did not change very much (see the main text, Figure 5). For comparison, we decided to analyze a situation when the protein will be turned to the ice block with its non-ice-binding surface. Therefore, we placed two proteins next to the two *secondary prism* planes of the ice block. One of these proteins faced the ice with the first non-ice-binding surface, while the other faced the ice with the second non-ice-binding surface of the *Cf*AFP molecule. On the *secondary prism* plane, only hexagonal ice was created, similarly as in the case of the *primary prism* plane.

In these two cases, we observed that the distance of these surfaces from the ice block, on average, changes more rapidly than in the case of the ice-binding surfaces facing the *basal* and *primary prism* planes of the ice block. This is illustrated in the main text (Figure 5). The distances were calculated as the distances of the planes passing through the alpha carbon atoms of the sheets constituting the ice-binding or non-ice-binding surfaces of the *Cf*AFP molecule from the appropriate surfaces of the ice block (specifically – planes passing through the most outer layers of oxygen atoms).

Below, we include the histograms of the distances of the ice-binding planes from the *basal* planes and *primary prism* planes of the ice block for 240 proteins from 120 simulations. On the histograms, we can see that although some proteins move farther than other, there is a stabilization or increase in the number of counts for the smaller distances for the *basal* and *primary prism* planes.

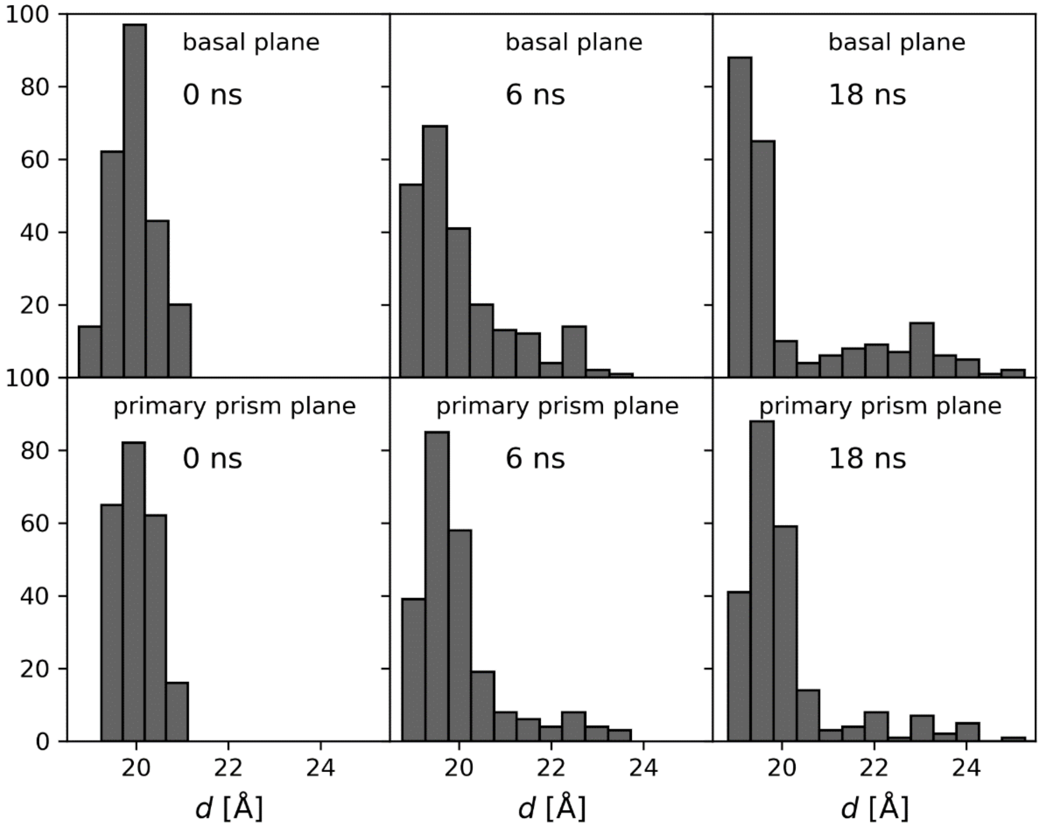

Figure S1. The histograms of the distances of the ice-binding planes from the *basal* planes and *primary prism* planes of the ice block for 240 proteins from 120 simulations (265 K). The histograms present the distances of the planes passing through the alpha carbon atoms of the sheets constituting the ice-binding surfaces of the *Cf*AFP molecule from the ice block surfaces. The axes are identical in all histograms.

2. The mutual arrangement of hydrogen-bonded water molecules characterized by the F4 parameter in the solvation shell of the ice block.

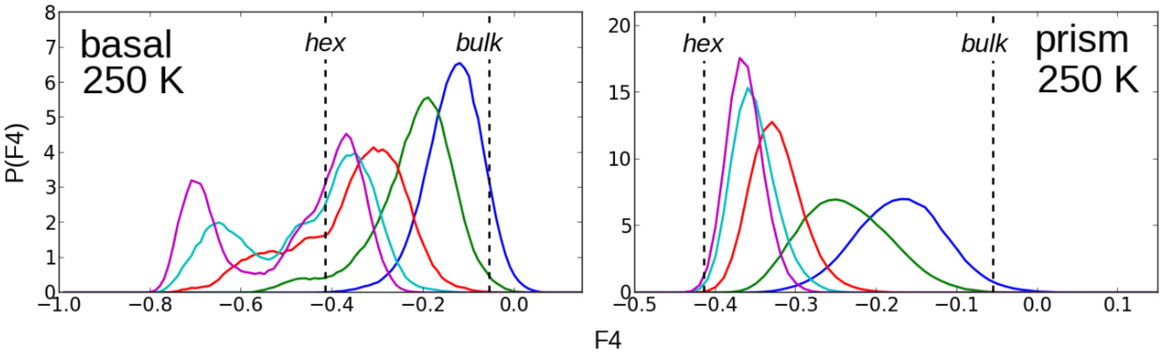

Figure S2. The average distribution of the F4 parameter for the solvation layer of the *basal* and *primary prism* planes of the ice block at different times: at the start of the simulation (blue line) and after 1.2 (green line), 6 (red line), 12 (cyan line) and 18 ns (magenta line). The calculations were conducted for the systems in which the molecules of CfAFP were present. The dotted black lines in the figure corresponds to the average values of the F4 parameter calculated for liquid water and hexagonal ice (denoted as "bulk" and "hex", respectively). The data presented here concerns the systems simulated at 250 K.

3. The energy of the interactions between a water molecule and its nearest surrounding.

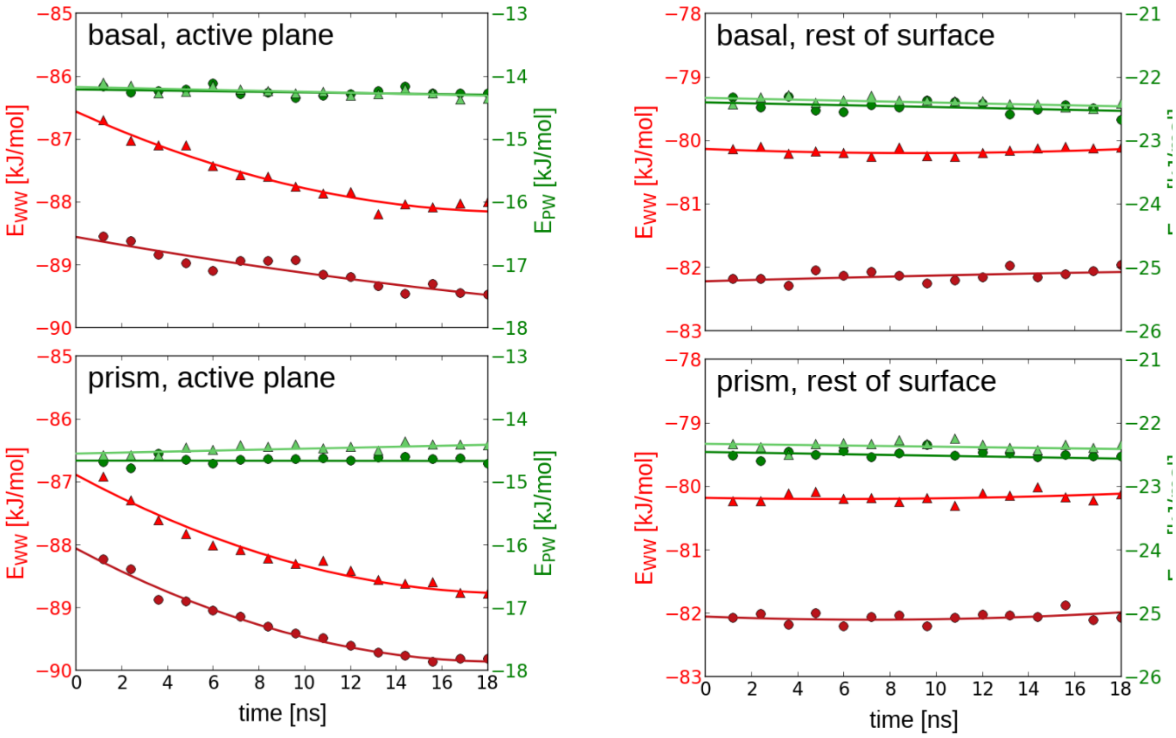

Figure S3. The changes of the energy of the interaction of water molecules located in the solvation shell of *Cf*AFP with the surrounding water (red markers) and the surface of the protein (green markers) in time. The energy of the interaction with the protein was calculated separately for the active (ice-binding) plane and the rest of the surface of the protein. The data presented here concerns the systems simulated at 250 K (dark red and dark green dots) and 265 K (light red and light green triangles).

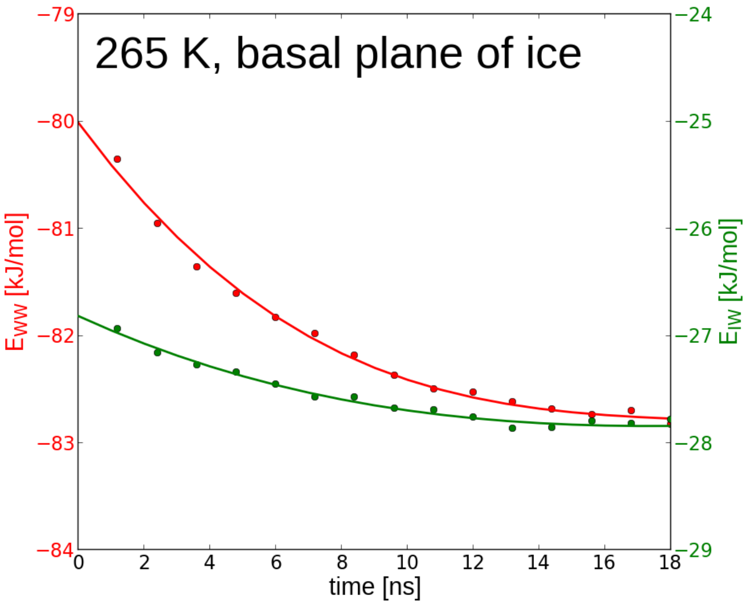

Figure S4. The changes of the energy of the interaction of water molecules located in the solvation shell of the *basal* plane of ice with the surrounding water (red dots) and the surface of the ice (green dots) in time. The data presented here concerns the systems simulated at 265 K. In the case of the systems simulated at 250 K the picture was very similar (data not presented).

4. The arrangement of the oxygen atoms in the crystal of ice.

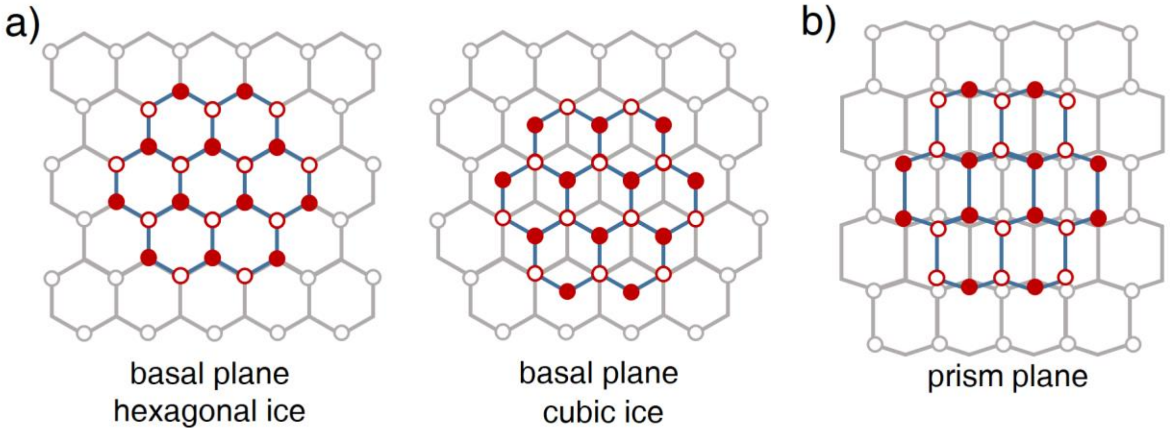

Figure S5. The arrangement of the oxygen atoms in the crystal of ice. The fragments of the structures of the crystallographic planes (*basal* and *primary prism*) of the investigated ice block are depicted in grey. In red, the arrangement of new ice molecules in the growing crystal is presented. At the surface of ice, there are two sublayers of water molecules: the first sublayer is outer (the oxygen atoms from this sublayer are indicated as circles with grey edges) and the second sublayer is inner (slightly below the first, hence not fully visible from the outside, its oxygen atoms are placed where the grey lines symbolizing hydrogen bonds connect).

The white circles with red edges and the full red circles correspond to the locations of the oxygen atoms of the water molecules that form the new layer of ice when the crystal grows (first sublayer, closest to the ice block – the white circles with red edges, second sublayer, a bit further from the ice block – full red circles).

a) At the *basal* plane, the oxygen atoms of water molecules locate themselves just above the most outer oxygen atoms of the ice (in the figure, the circles with red edges cover the circles with grey edges). With these molecules, molecules marked as the full red circles connect. In the figure, the full red circles cover the locations of the oxygen atoms from the inner sublayer of the ice block surface or are located above the centers of the hexagons. These two possible arrangements of the second sublayer of oxygen atoms correspond to the hexagonal or cubic structure of ice, respectively. Hydrogen bonds between molecules from the two sublayers are depicted as blue lines. b) On the *primary prism* plane of ice, oxygen atoms of the new layer of ice (the white circles with red edges and the full red circles) are not located exactly above the oxygen atoms in the inner and outer sublayer at the surface of the ice block – they are a bit shifted.

5. The adjustment of the hydroxyl oxygen atoms of the threonine residues of the protein to the ice lattice.

The mutual adjustment of the protein and ice was investigated by calculating the standard deviation of the coordinates of the hydroxyl oxygen atoms of the threonine residues of the protein projected on the surface of ice crystal from the coordinates of the most outer oxygen atoms that forms the ice lattice. The details of the calculations were as follows.

The analysis was done for the proteins next to both *basal* planes and next to both *primary prism* planes of the ice block. At the ice-binding plane of the *Cf*AFP molecule there are nine threonine residues, arranged in two rows, 4 and 5 residues long (Figure S6 A and B). The coordinates of the hydroxyl atoms of these nine residues were projected onto the plane passing through the most outer layer of the oxygen atoms at the surface of the ice block. Next, the outer oxygen atom of the ice closest to the projected position of each hydroxyl oxygen of the threonine was identified. Then, the standard deviation of these nine points from the reference positions (coordinates of the oxygen atoms at the ice surface) was calculated.

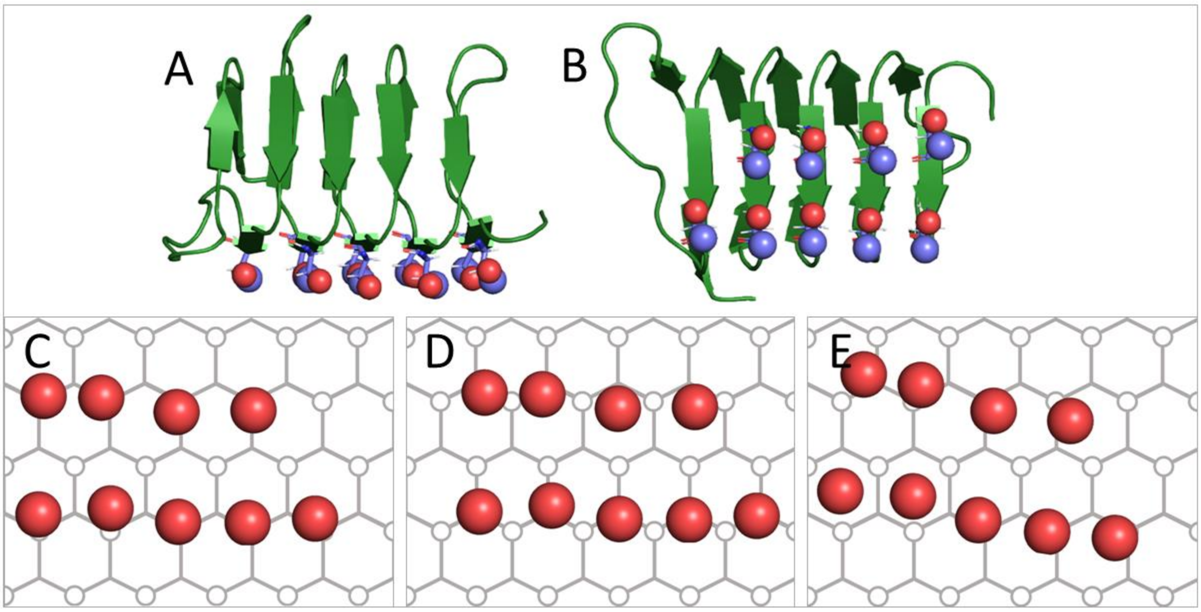

Figure S6. The mutual adjustment of the oxygen atoms of the threonine residues of the protein and the ice lattice. A side view on the *Cf*AFP molecule (A) and a front view on the active plane of the protein (B) - threonine residues of the active site of the protein are shown as sticks, oxygen atoms of the hydroxyl groups and carbon atoms of the methyl groups of the threonine residues are marked as red and blue spheres, respectively. One of the possible orientations of the oxygen atoms of the active plane of the protein relative to the ice lattice is that the oxygen atoms of the protein (red spheres) are located near the positions of the oxygen atoms of water molecules of the most outer layer of the ice crystal (marked as white circles with gray edges) (C). The value of the standard deviation calculated for this set of points is lower than in the case of random distribution. On the other hand, oxygen atoms of the protein could also place themselves above the oxygen atoms of the water molecules of the inner sublayer of the ice surface (D) or above the middles of the hexagons (compare with Figure S5a). In those cases, the value of the standard deviation would be larger than for the random distribution. The orientation of the oxygen atoms of the protein relative to the ice lattice can also be random (E), which gives the values of the standard deviation presented as the black dotted line in Figure 6 in the main text.

The analysis of the selected fragment of the trajectory gave us a histogram of the distribution of these standard deviations. The results for all 240 proteins in 120 copies were collected together to describe the average behavior of the system and they are presented in the main text. For comparison, we also prepared theoretical histograms of these mean standard deviations if there were absolutely no orientational preferences (*i.e.* if the positions of the hydroxyl oxygen atoms were random).

As it can be seen in the Figure 6 in the main text, the obtained histograms change in time and they are different from the random distribution. The mutual adjustment of the protein and ice becomes better as the simulation progresses – it is represented on the histograms as increasing amount of values of the standard deviation that are smaller or larger than in random distributions. Smaller values of the mean standard deviations correspond to the situation, when the projected positions of the oxygen atoms located at the surface of the ice-binding plane of protein are close to the oxygen atoms on the most outer layer of ice crystal (Figure S6 C), while the larger values correspond to the situation, when the oxygen atoms of the protein are at larger distances from the reference points compared to the random distribution (Figure S6 D). One example of a random position is presented in Figure S5 E - the projections of the coordinates of the oxygen atoms of the protein onto the *basal* plane could be as marked by the full red circles.