Supplemetary information of : Antifreeze proteins and homogeneous nucleation: on the physical determinants impeding ice crystal growth

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AFP MODEL AND SIMULATION DETAILS

In this section we present the square protein on a square lattice model for a protein anchoring an ice slab, following the model proposed by Raymond and DeVries [1]. The AFPs are characterized by a series of structures, according to the regnum (animalia, plantae, fungi, bacteria) they have been observed [2], whose ice-controlling activity varies substantially. Some works [3–6] suggested that the AFPs enhance the formation of a "quasi-ice layer" on the water hydrating the protein ice-biding surface, although recent data show that the ice ordering of water near the protein surface is quite low [7] and that the protein's effect on the hydration water consists in slowing down the hydrogen bond (HB) dynamics [8–10]. Moreover, recent evidences support the idea that the AFP binds irreversibly the ice surface [11].

To simplify the analysis of the relevant factors affecting the ice growth we model the AFP as a wall of Argon-like particles, tangent each other and arranged as a fcc lattice. The AFP sizes are $l \times l \times h_{AFP}$, with l = 2.5 nm and $h_{AFP} = 1.2$ nm. The protein surface anchoring the ice is covered with an ice layer, considered part of the protein structure, to ensure the ice-protein match. Fig. 1a shows an example of the initial setup with an ice block sandwiched between two AFPs: the larger atoms constitute the body of the AFP, while the blue atoms are the anchoring region of the AFP stably bound to the ice. To guarantee the irreversible ice-AFP binding [12, 13] the protein structure is kept frozen along the simulation.

The AFP is anchored on the basal plane of the ice crystal. According to Espinosa et al. [14] the surface free energy for the basal, primary prismatic, and secondary prismatic planes of the TIP4P/Ice water model are, respectively, 27.2, 31.6 and 30.7 mJ/m². Those values refer to a flat solid-liquid interface, which represents the equilibrium interface only at the coexistence line. Nonetheless, if the interface is curved, the surface free energy is $\gamma_{sl} = 30.00[1 - 0.24(n - 1)/(Rc/nm)] \text{ mJ/m}^2$ [15, 16], with n = 3 for a spherical interface and n = 2 for a cylindrical interface. As the value of the critical radius R_c at each T is unique, it is also possible to express γ_{sl} as a function of temperature which, for the TIP4P/Ice model, adopts the form $\gamma_{sl}/(\text{mJ/m}^2) = 30 - 0.2748(T_m - T)$ (valid for both the spherical and the cylindrical interfaces). Thus, assuming the AFP-ice bound to be irreversible, the value γ_{sl} for the equilibrium solid-liquid interface for any $\Delta T > 0$ depends only on the curvature radius R_c of the plane to which the AFP is bound.

Any atom of the AFP's body interacts with water via Lennard-Jones potential $U(r) \equiv 4\epsilon [(\sigma/r)^{12} - (\sigma/r)^6]$, with depth of $\epsilon = 0.5$ kJ/mol and $\sigma = 0.31668$ nm. This makes the protein surface exposed to the water slightly hydrophobic, according to what observed for the surface composition of that ice-binding proteins [17, 18].

The proteins and the ice slab are immersed in a bulk of supercooled liquid water. Periodic boundary conditions (pbc) apply to the three directions. We fix the initial condition of the simulation box with $L_x \sim L_y$ according to the ice structure.

Simulations are performed with GROMACS [19, 20] at pressure p = 1 bar in the anisotropic NpT ensemble with a time step of 2 fs, letting the XYZ dimensions to fluctuate separately to accommodate the crystal structure, and changing the temperature below the freezing point of the water model $T_f = 270$ K[21]. We adopt the Noose-Hoover thermostat, with a relaxation time of 1 ps, and the Parrinello-Rahman barostat wit a relaxation time of 2 ps. d is not fixed, but fluctuates according to the variation of L_x and L_y sizes. Along the simulation we monitor that $L_x \sim L_y$ fluctuate slightly. PME of order 4, with fourier spacing of 0.1, is used to solve the electrostatic interaction. The cutoff is 0.9 nm both for the Lennard-Jones and for the real space part of the Coulombic interactions. Long range corrections to the Lennard-Jones ineractions were included.

Along the simulations the number of ice molecules is calculated according to the $\overline{q_6}$ order parameter [22], adopting a cutoff distance of 3.5 Å, and considering solid the molecules whose $\overline{q_6} > 0.37$.

In the Fig. 1b-d we report three snapshots of the ice spanning into the supercooled liquid water and embracing the AFP. As observed also in Ref. [23], below the stability limit of the solid-liquid interface the ice spans and the AFP is rounded by ice that tends to bridge from different sizes of the AFP.

It is clear that, once the solid-liquid interface is no longer stable because the system is cooled down, at the equilibrium all water molecules crystallize, with a certain degree of order/disorder close to the AFP. Nevertheless, the average time required to crystallize the entire system could depend on the AFP-water interaction. Indeed, in our simulations we observed changes in the ordering of the water molecules rounding the AFP and the time required to bridge two sides of the AFP according to the adopted AFP-water potential. For example, a strong increase of the isotropic interaction caused a local melt of the ice in contact with the AFP interface forcing the solid to bridge at further distance from the AFP. Also, imposing a directional and disordered AFP-water potential resulted in a local distortion of the ice ordering at the AFP interface, slowing down the nucleation rate. A systematic study of these effects was beyond the scope of the present manuscript and will be characterized in future works.

In Fig. 2 we report the data referring to all the AFP-AFP distances d simulated.



FIG. 1. AFPs separated by a distance $d_{\text{max}} \sim 5.4$ nm and with N = 17584 water molecules. (a) Initial configuration of two AFP anchored on both sides of the ice slab. (b-d) Ice growing and wrapping the AFPs at T = 263 K, at times ~ 180 ns (a), ~ 220 ns (b) and ~ 320 ns (c). These configurations are taken along the trajectory shown with cyan color in Fig. 2 of the main manuscript. For all panels Z direction runs from left to right.



FIG. 2. (a) Time evolution of the fraction of ice molecules, for different temperatures T. The total number of water molecules is N = 12929, with $d \sim 4.35$ nm. (b) N = 23701 water molecules with AFPs separated by $d \sim 6.5$ nm. (c) N = 22729 water molecules with AFPs separated by $d \sim 7.1$ nm. (d) N = 37680 water molecules with $d \sim 7.8$ nm. (e) N = 45425 water molecules with $d \sim 10.6$ nm. (f) N = 120768 water molecules with $d \sim 16.3$ nm.



FIG. 3. Supercooling ΔT^{max} as function of the protein-protein distance *d*. Black points are the outcomes of our simulations. Blue dashed line is the fit $\Delta T^{\text{max}} = 4476.52/d^4$. The remaining lines are the predictions according to the original Reymond and DeVries theory for spherical and cylindrical interfaces, assuming γ as a constant (solid lines), or an improved version of the theory of Raymond and deVries (dotted lines) where γ_{sl} depends on the temperature according to $\gamma_{sl}/(\text{mJ/m}^2) = 30 - 0.2748(T_m - T)$, ρ_{ice} depends on *T* and the $\Delta\mu$ is evaluated exactly for the model and not under the approximation $\Delta\mu = \Delta H_m \Delta T/T_m$. This improved version is obtained by solving (for *T*) Eq.(12) for each value of *d* (using $\cos(\theta) = 1$) and using the exact values for the water model of $\rho_{\text{ice}}(T)$, $\Delta\mu(T)$ and $\gamma_{sl}(T)$ [15, 16].

CLASSICAL NUCLEATION THEORY AND AFP EFFECT

A simple thermodynamic approach to derive the equilibrium curvature of a solid-liquid interface relies on the fact that, at the equilibrium, the chemical potential of the solid and liquid phases, indicated respectively with μ_{sol} and μ_{liq} , must be equal

$$\mu_{\rm sol}(p_{\rm sol}) = \mu_{\rm liq}(p_{\rm liq}) \qquad (1)$$

Across the curved interface there is a different pressures between the liquid and the solid phases, respectively p_{sol} and p_{liq} , such that

$$\mu_{\rm liq}(p_{\rm liq}) = \mu_{\rm sol}(p_{\rm liq}) + \int_{p_{\rm liq}}^{p_{\rm sol}} v_{\rm sol} dp \qquad , \tag{2}$$

being v_{sol} the volume of the solid phase. The solid phase can be reasonably assumed to be incompressible, therefore Eq. (2) can be read as

$$\Delta \mu \equiv \mu_{\rm liq}(p_{\rm liq}) - \mu_{\rm sol}(p_{\rm liq}) = v_{\rm sol}(p_{\rm sol} - p_{\rm liq}) = v_{\rm sol}\Delta p \qquad (3)$$

Applying Laplace's equation $\Delta p = 2\gamma_{sl}/R_c$ for a spherical interface and $\Delta p = \gamma_{sl}/R_c$ for a cylindrical interface, where γ_{sl} is the solid-liquid surface free energy. By replacing Δp in the previous expression and substituting $v_{sol} = \rho_{ice}^{-1}$, where ρ_{ice} is the number density, we get

$$\Delta \mu = \frac{2\gamma_{sl}}{R_c \rho_{\rm ice}} \qquad . \tag{4}$$

The Eq. (4) can be easily solved for a spherical or cylindric interface, resulting in

$$R_{c} = \frac{2\gamma_{sl}}{\rho\Delta\mu} \quad \text{for spherical interface}$$

$$R_{c} = \frac{\gamma_{sl}}{\rho\Delta\mu} \quad \text{for cylindric interface} \quad , \tag{5}$$

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which, under the approximation $\Delta \mu \sim \Delta H_m \Delta T/T_m$, read as the expression proposed by Raymond and DeVries [1], and reported also in Eq. (12). Eq.s (5) express the curvature radius of a spherical and cylindrical interface, as long as the mechanical stability holds.

The result of Ref. [1] can be get also following the work of Naullage, Qiu and Molinero [23]. According to the authors, the free energy associated to the formation of a solid-liquid interface is

$$\Delta G = -\Delta \mu V_d \rho_{\rm ice} + \gamma_{sl} (A_d - A_{\rm flat}) + \tau \ell, \tag{6}$$

where ρ_{ice} is the number density of the ice; τ is the line tension; ℓ the length of the AFP-solid-liquid contact line. In our simulations, the solid-liquid interface resemble a sphere surface along the larger distance d, shown in Fig. 1 of the main manuscript. This interface merges with its pbc replica via quasi-cylindrical interface, with section d'.

For a spherical interface, V_d is the volume of the spherical cap of proteins separated by a distance d

$$V_d = \pi h^2 \left(R_c - \frac{h}{3} \right) = \frac{\pi d^3}{8 \cos^3 \theta} (1 - \sin \theta)^2 \left[1 - \frac{1}{3} \left(1 - \sin \theta \right) \right] = \frac{\pi d^3}{24 \cos^3 \theta} (\sin^3 \theta - 3 \sin \theta + 2) \qquad . \tag{7}$$

 A_d is the surface of the cap

$$A_d = 2\pi R_c h = \frac{\pi d^2}{2\cos^2\theta} (1 - \sin\theta) \qquad , \tag{8}$$

 A_{flat} is the flat surface replaced by the cap

$$A_{\text{flat}} = \frac{\pi d^2}{4} \qquad . \tag{9}$$

By replacing the expression for V_d , A_d and A_{flat} into Eq. 6 we get

$$\Delta G_{\rm sphere} = -\Delta \mu \rho_{\rm ice} \frac{\pi d^3}{24 \cos^3 \theta} (\sin^3 \theta - 3 \sin \theta + 2) + \frac{\gamma_{sl} \pi d^2}{2} \left[\frac{1 - \sin \theta}{\cos^2 \theta} - \frac{1}{2} \right] + \tau \ell. \tag{10}$$

Alike, following Ref. [23], we get the expression $\Delta G_{\text{cylinder}}$ in the case of cylindrical interface. In such a case, the AFP proteins are separated by a distance $d' \equiv d/\sqrt{2}$, and by replacing in Eq. (6) $V = d'^2 l(\pi - 2\theta - \sin 2\theta)/(8\cos^2\theta)$, $A_d = d' l(\pi - 2\theta)/(2\cos\theta)$ and $A_{\text{flat}} = d'L$,

$$\Delta G_{\text{cylinder}} = -\Delta \mu \rho_{\text{ice}} d'^2 l \frac{\pi - 2\theta - \sin 2\theta}{8\cos^2 \theta} + \gamma_{sl} d' l \left(\frac{\pi - 2\theta}{2\cos \theta} - 1\right) + 2\tau l \qquad (11)$$

Note that for the cylindrical interface the new AFP-solid-liquid contact line is twice the X (or Y) size of the AFP protein l.

The minimum of the free energy satisfies $\partial \Delta G / \partial \theta = 0$, which results in the condition

$$\frac{\cos\theta}{d} = \frac{\Delta\mu\rho_{\rm ice}}{4\gamma_{sl}} = \frac{1}{2R_c} \qquad \text{for the spherical interface;} \\ \frac{\cos\theta}{d} = \frac{\Delta\mu\rho_{\rm ice}}{2\gamma_{sl}} = \frac{1}{2R_c} \qquad \text{for the cylindric interface,} \tag{12}$$

where $R_c = d/(2\cos\theta)$ is the curvature radius of the interface (Fig. 1 of the main manuscript). By approximating $\Delta\mu \sim \Delta H_m \Delta T/T_m$ Eq.s (12) recover the expression of the Gibbs-Thomson effect, proposed by Raymond and DeVries [1] and reported in Eq. (1) of the manuscript. Nevertheless, as shown in Fig. 4, such an approximation for $\Delta\mu$ does not work properly for the TIP4P/Ice water model for ΔT larger than few degrees.

We can neglect the terms depending on τ in both the expression for ΔG of Eq.s (10) and (11), as also reported in Ref. [23]. In Fig. 5 we plot both ΔG_{sphere} and $\Delta G_{\text{cylinder}}$ for two cases where the ice clearly grows in our simulations, i.e. for T = 263 K and $d \sim 5.4$ nm, and for T = 253 K and $d \sim 4.1$ nm (both shown in Fig. 2 of the manuscript). As shown, both Eq.s (10) and (11) predict a stable solid-liquid interface with a contact angle $\theta > 0$ and, in three cases, huge free energy barriers which are not compatible with our simulation findings. The free energy barrier for the cylindrical interface with $d \sim 4.1$ nm and T = 253K is $\sim 7k_BT$ (red curve in right panel of Fig. 5): not huge.



FIG. 4. $\Delta \mu \equiv \mu_{\text{liq}} - \mu_{\text{sol}}$ for the TIP4P/Ice water model (black curve) and its approximation according to the relation $\Delta \mu = \Delta H_m \Delta T/T_m$ (red dashed line), where the melting enthalpy of the TIP4P/ice model is $\Delta H_m = 1.29$ kcal/mol.



FIG. 5. ΔG_{sphere} (left panel) and $\Delta G_{\text{cylinder}}$ (right) panel) curves predicted respectively by Eq.s (10) and (11) for AFPs separated by: $d \sim 5.4$ at T = 263 K (black curve); $d \sim 4.1$ at T = 253 K (red curve). Insets zoom on the region of local minimum.



FIG. 6. ΔG_{sphere} according to Eq. (10) for AFPs separated by $d \sim 5.4$ at different T. Black circles mark the minimum of each curve.

Both the expression for ΔG_{sphere} and $\Delta G_{\text{cylinder}}$, although predict an minimum corresponding to an angle $\theta > 0$

and decreasing with T for any stable solid-liquid interface, imply that the ice spans only when $\theta = 0$ (or $\theta \leq 5^{\circ}$ to which correspond a free energy barrier of $\sim 1k_BT$). In the Fig. 6 we report as example the behavior of ΔG_{sphere} for a specific value $d \sim 5.4$ nm, and different supercooling ΔT .

In conclusion, the straight forward application of Eq.s (10) and (11), although nicely predicts the value contact angle θ for the stable interfaces as proven in Ref. [23] for a cylindrical interface and in Fig. 5 of the manuscript for a spherical interface, cannot predict, at least for a supercooling $\Delta T < 13$ K, the supercooled limit of stability of such interfaces.

POSSIBLE EXPLANATION FOR THE SPANNING POINT OF ICE

We assume that the AFPs prevent the ice growth as long as the ice's height (with respect to the plane where the AFP is anchored on top of the ice) protruding into the supercooled liquid does not exceed the height of the AFP. According to our simulations, for any supercooled T at which the crystal-liquid interface is stable, we observe the formation of a cylindrical interface along the X and Y directions, and spherical interface along the diagonal rising on top of the cylindrical one (Fig. 3 of the main text and Fig. 7 of the S.M.). If we denote with $h_{\rm cil}$ and $h_{\rm sp}$ the heights of the cylindric-like and sphere-like interfaces respectively, the control parameter for the ice growth should be

$$h_{\rm cil} + h_{\rm sp} = R_{\rm cil}(1 - \sin\theta_{\rm cil}) + R_{\rm sp}(1 - \sin\theta_{\rm sp}) \tag{13}$$

being $R_{\rm cil}$ and $R_{\rm sp}$ the curvature radius for the cylindric-like and sphere-like interfaces according to the CNT (see Eq. 12), respectively, and $\theta_{\rm cil}$ and $\theta_{\rm sp}$ the contact angles. Replacing Eq. (12) into the previous expression and considering that $R_{\rm cil} = d/(2\sqrt{2}\cos\theta_{\rm cil})$ and $R_{\rm sp} = d/(2\cos\theta_{\rm sp})$ we get

$$h_{\rm cil} + h_{\rm sp} = R_{\rm cil} \left(1 - \sqrt{1 - \frac{d^2}{8R_{\rm cil}^2}} \right) + R_{\rm sp} \left(1 - \sqrt{1 - \frac{d^2}{4R_{\rm sp}^2}} \right) = \frac{\gamma_{sl}}{\Delta\mu\rho_{\rm ice}} \left[3 - \sqrt{1 - \left(\frac{d\Delta\mu\rho_{\rm ice}}{2\sqrt{2}\gamma_{sl}}\right)^2} - 2\sqrt{1 - \left(\frac{d\Delta\mu\rho_{\rm ice}}{4\gamma_{sl}}\right)^2} \right]$$
(14)

where $\Delta \mu$, ρ_{ice} and γ_{sl} depend on T. The variation of $\Delta \mu$ with T is shown in Fig. 4 of this supplementary material. The variation of γ_{sl} with T is also provided above in this Supplementary material. Finally, the number density of ice ρ_{ice} can be obtained easily from the mass density of ice for the TIP4P/Ice model which is well described by the expression $\rho_{ice}/(g/cm^3) = 0.906 - 0.00015(T - T_m)$.

We observed in our simulations that the ice growth was inevitable anytime the height of the ice meniscus, given by $h_{\rm cil} + h_{\rm sp}$, was larger than the height of the AFP $h_{\rm AFP} = 1.2$ nm. By imposing $h_{\rm cil} + h_{\rm sp} = h_{\rm AFP}$ we get a relation between d and the supercooled T via $\Delta \mu$, $\rho_{\rm ice}$ and γ_{sl} , that establish the lowest T where the height of the ice interface matches the AFP height. The curve is shown with a black line Fig. 6b of the main manuscript.

As described in the main text, this estimation can be further improved by including the ice fluctuation via the Zeldovich factor (red line in Fig. 6b of the main manuscript).

SOLID-LIQUID INTERFACE, CURVATURE RADIUS R_c AND CONTACT ANGLE θ

Along the simulations we labeled the water molecules as liquid or solid according to the $\overline{q_6}$ order parameter. For the run where the crystal-liquid interface was stable, after dividing the X - Y planes in regular bins, we computed the average Z coordinates per bin of the highest and lowest crystal molecules along the Z direction (which is the direction where the ice spans). By construction, this defines the average position of the crystal-liquid interfaces (one for each AFP). In Fig. 7 we show the upper average interfaces for AFP separated by $d \sim 4.1$ nm.

The crystal-liquid interface resembles a merge between a sphere and a cylinder, as previously mentioned. To compute the curvature radius of the sphere, after computing the average ice interface (Fig. 7), we cut it with the planes $X = 3\langle L_x \rangle/4$ and $Y = 3\langle L_y \rangle/4$, schematically indicated by the red arrows of Fig. 8a. The curve given by this intersection is sketched with black dots in Fig. 8b, where we can distinguish the projections of the cylindrical (on the left – quasi-constant dots) and spherical (on the right) interfaces. This rough estimation of the projection of the spheric interface allow to compute the distance D and the height h, as shown in 8b. Note that we have two crystal-liquid interfaces, and each is cut along this planes; therefore we estimate D and h from any projection. Finally, given the a simple relation $(R_c - h)^2 + (D/2)^2 = R_c^2$ that connects the curvature radius R_c with h and D, we compute $R_c = (D^2 + 4h^2)/(8h)$ for any projection, and than average it over the four projections.



FIG. 7. Ice surface in a system with N = 13130 water molecules, separated by a distance ~ 4.1 nm, for (a) T = 256K, (b) T = 258K, (c) T = 260K, (d) T = 262K, (e) T = 264K, (f) T = 266K.

The curvature radius is finally compared with the radius of the ice critical nucleus. In particular, from the works of Espinosa et al. [24] and Niu et al. [25] we estimated the *T* dependence of $R_c(T)$ on the size of critical nucleus N_c as $R_c(T) = [3N_c(T)v^{ice}/(4\pi)]^{1/3}$, being $N_c(T)$ the *T*-dependent size of the critical ice nucleus, and $v^{ice} \sim 0.033$ nm³ the ice volume per molecule for the TIP4P/Ice model (v^{ice} decreases with *T* of ~ 0.7% within the range of temperatures [230 : 270] K and can be assumed constant for our calculation). The values are reported in Fig. 6a of the main text. From R_c we estimate θ for the spheric interface via the relation $\cos \theta = R_c/(d/2)$ (see Fig. 1c of the main text).



FIG. 8. Scheme showing the procedure to compute the curvature radius. We project the ice surface ridge onto the XZ and YZ planes. The ice ridge is indicated by red arrow in the left panel, parallel to the X and Y axes and passing through the highest point of the ice dome. From any projection, sketched in the right panel, we compute the height of the dome h and its base D (left panel). D is the effective base of the meniscus calculated along the X (or Y) direction.

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