

## Kinetic Pinning and Biological Antifreezes

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(Received 12 April 2004; published 15 September 2004)

Biological antifreezes protect cold-water organisms from freezing. An example is the antifreeze proteins (AFP's) that attach to the surface of ice crystals and arrest growth. The mechanism for growth arrest has not been heretofore understood in a quantitative way. We present a complete theory based on a kinetic model. We use the "stones on a pillow" picture. Our theory of the suppression of the freezing point as a function of the concentration of the AFP is quantitatively accurate. It gives a correct description of the dependence of the freezing point suppression on the geometry of the protein, and might lead to advances in design of synthetic AFP's.

DOI: 10.1103/PhysRevLett.93.128102

PACS numbers: 87.14.Ee, 68.08.-p, 81.10.Aj

In polar regions, many fish, insects and plants flourish at temperatures well below the freezing point of their bodily fluids [1–4]. Often, particularly in fish [5], this is a noncolligative effect; ice growth within the organisms is arrested by a class of plasma proteins called antifreeze proteins (AFP). They are usually peptides or glycopeptides. These molecules attach irreversibly to ice surfaces [5] and prevent crystal growth [1,6] until the water containing the AFP's is supercooled by as much as two degrees Celsius. The mechanism for the suppression of the freezing point is not understood. In this Letter we present a growth model which allows us to understand AFP's in considerable detail, and, in particular, to calculate the dependence of the undercooling on AFP concentration. [7,8].

Growth arrest by AFP's is known to occur because the protein adsorbs on the surface of the growing ice crystal, and suppresses growth near that site. This could stop growth in various ways. One picture is that the AFP's are obstacles to step-flow growth [5] of a facet. However, such a mechanism assumes that the surface is faceted, which is true only for a basal plane of ice near the freezing point. Other surfaces of ice are thermally rough at those temperatures, and AFP's adsorb mostly on those surfaces [6]. An alternative picture, which we adopt, is the "stones on a pillow model" of Knight *et al.* [1,2]. In that view, the AFP's are obstacles to growth of a smoothly curved surface. To grow, the crystal must bulge between the attachment sites, and the freezing point is depressed by the Gibbs-Thompson effect, namely, that a curved surface has a lower freezing point than a flat one; see Fig. 1(a). Here, we present a quantitative kinetic theory of biological antifreezes based on this picture.

The surface of the crystal which is not under the AFP must have constant mean curvature  $\kappa$  given by the Gibbs-Thompson condition [9]:  $\kappa = -\delta_T/l_o$  where  $\delta_T = (T_m - T)/T_m$  (the dimensionless undercooling) and  $T_m$  is the equilibrium melting temperature [9].  $l_o$  is a characteristic length, namely, the interfacial tension  $\gamma$  divided by  $\Lambda$ , the

latent heat of fusion per unit volume. For ice  $l_o \approx 1 \text{ \AA}$ . Consider a surface  $h(x, y)$  which has stopped growing. We assume that the AFP's prevent growth in a circle of radius  $b \sim 10 \text{ \AA}$  on the surface. This would be the case if the AFP were spherical and partly buried in the ice, for example. (We treat more complicated molecular geometries, below.) The angle of the ice with the blocked region can take on any value. In the small-slope approximation,  $|\partial h/\partial x| \ll 1$ ,  $|\partial h/\partial y| \ll 1$ , the curvature of the interface is given by  $\kappa \approx \Delta h$ . Then the Gibbs-Thompson condition becomes the Poisson equation:

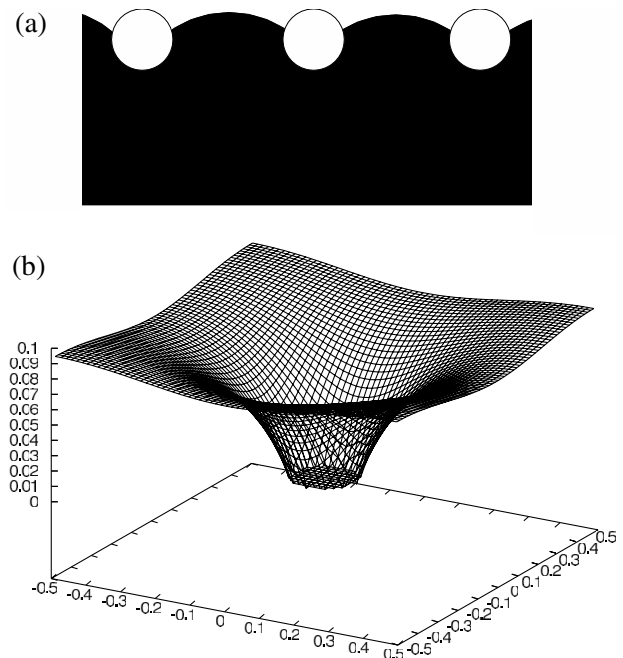


FIG. 1. (a) The "stones on a pillow" model. AFP molecules, white circles, attach to a surface. (b) Visualization of a surface of constant mean curvature. The size of the unit cell has been set to unity.

$$\Delta h = -\delta_T/l_o \equiv -4\pi\rho. \quad (1)$$

This equation is familiar in electrostatics.  $h$  plays the role of the potential, and the effective (positive) charge density  $\rho$  is defined by Eq. (1).

Now consider a periodic square array of AFP's. We must solve Eq. (1) with boundary conditions that the normal derivative of  $h$  vanishes at the edge of the unit cell, and  $h = 0$  at the edge of the AFP. The problem is easily solved numerically; a contour plot is shown in Fig. 1(b). In order for a periodic solution to the equation to exist, the AFP must act as a negative charge so that the system is "neutral", i.e., flat on large scales. That is,  $\rho = -nq$ , where  $n$  is the density of AFP's on the surface. To define the effective charge  $q$  we integrate Eq. (1) over the surface and use Gauss' law:

$$\langle(\mathbf{r} - \mathbf{r}_i) \cdot \nabla h\rangle_{|\mathbf{r}-\mathbf{r}_i|=b} = \delta_T/(2\pi l_o n) \quad (2)$$

where  $\langle \cdot \rangle$  is the average around the edge of the AFP. The charge represents the slope of  $h$  at the edge of the AFP:

$$q = -(1/2)\langle(\mathbf{r} - \mathbf{r}_i) \cdot \nabla h\rangle_{|\mathbf{r}-\mathbf{r}_i|=b} \quad (3)$$

so that  $|q| \approx (b/2)(\partial h/\partial r)$ .

From Eq. (2), the slope at the edge of the AFP increases with undercooling. We assume that if  $\partial h/\partial r$  at the edge of the AFP exceeds some critical value  $\chi$  the anti-freeze molecule will be engulfed.  $\chi$  is set by the physical chemistry of the AFP and the interface, and should be of order unity or less. Now, the maximum undercooling  $\delta_T^*$  is given by Eq. (2) with  $\langle \partial h/\partial r \rangle = \chi$ . For  $b \sim 10 \text{ \AA}$ , and  $\delta_T^*$  corresponding to  $1^\circ$  undercooling we need  $n = n^* = \delta_T^*/(2\pi\chi l_o b) \sim 10^{12} \text{ cm}^{-2}$ , or a distance between AFP's of order  $100 \text{ \AA}$ . From Eq. (3) we find  $|2q| < \chi b$ .

For irreversible adsorption, the relationship between  $n$ , the density of AFP on the surface, and the concentration in solution must depend on the kinetics of the growth of the crystal, which we now model. In linear response theory, the growth speed of the crystal boundary is proportional to the variation of the overall free energy of the system with respect to the normal displacement. In the small-slope limit we take this to be  $\delta h$ . Then

$$\dot{h} = -\Gamma \frac{\delta}{\delta h} \int [\gamma(1 + |\nabla h|^2)^{1/2} + \Lambda \delta_T h] d^2\mathbf{r}. \quad (4)$$

The first term of the integrand is the free energy per unit area of surface, and in the second,  $\Lambda \delta_T$  is the free energy difference of solid and liquid phases per unit volume close to melting point.  $\Gamma$  is a kinetic coefficient, such that  $v_o = \Gamma \Lambda$  is the growth velocity of a flat surface at unit undercooling. This equation is equivalent to [10]

$$\dot{h} = v_o[l_o \Delta h + \delta_T]. \quad (5)$$

The important parameters for growth are a velocity  $v_o$ , a length  $l_o$ , and  $\delta_T$  (dimensionless).

The AFP's are pinning centers for the surface, whose effect may be expressed as a set of boundary conditions:

$$h|_{|\mathbf{r}-\mathbf{r}_i|=b} = h_i. \quad (6)$$

Here  $\mathbf{r}_i$  is the position of  $i$ th AFP and  $h_i$  is the local position of the interface when the  $i$ th AFP is adsorbed. We assume that the AFP's in the water adsorb at random on the ice surface with rate,  $k_+$ , per unit area.

It will be useful to split  $h(\mathbf{r})$  into two parts:  $h(\mathbf{r}) = H + \phi(\mathbf{r})$ , where  $H$  is the average height, and  $\phi(\mathbf{r})$  measures the small-scale variations. The AFP's have density  $n$ . We choose  $\phi$  to develop in time as

$$\dot{\phi} = v_o l_o (\Delta \phi + 4\pi\rho) \quad (7)$$

where  $\rho = n\langle q \rangle$  is chosen, as above, to make the surface flat on the average, and  $\langle q \rangle$  is the average charge. It is not hard to show that the time derivative in Eq. (7) for  $\phi$  is small (the quasistatic limit). Thus

$$\Delta \phi = -4\pi[\rho + \sum_i q_i \delta(\mathbf{r} - \mathbf{r}_i)]. \quad (8)$$

The last term accounts for the boundary conditions, Eq. (6). The slopes at the edge of the AFP's are not fixed: as the interface moves the slopes increase from 0 at the moment of adsorption to  $\chi$ , at which point the AFP is engulfed by ice. Correspondingly,  $0 < |q| < \chi b/2$ .

Near an AFP,  $\phi$  is dominated by the contribution of that protein. Electrostatics in two dimensions gives [11]

$$\phi(\mathbf{r}) \simeq -q_i \log[\pi(\mathbf{r} - \mathbf{r}_i)^2 n]. \quad (9)$$

Therefore,

$$q_i \simeq \frac{H - h_i}{\log(\pi n b^2)} < 0. \quad (10)$$

Suppose the interface moves with constant speed  $V$ . Each  $h_i$  is fixed, but  $H$  changes uniformly with time. Equation (10) implies that the magnitudes of individual point charges  $q_i$ , and thus the slopes at the edges of the AFP's, are uniformly distributed between 0 and  $-\chi b/2$ , i.e.,  $\langle q \rangle = -\chi b/4$  and  $\rho = nb/4$ .

Subtracting Eq. (7) from Eq. (5), we find  $\dot{H} = v_o[\delta_T - 4\pi l_o \rho]$ . If  $\dot{H} = V$ , we have  $\rho = nb/4$ , and

$$V = v_o(\delta_T - \pi\chi l_o b n). \quad (11)$$

The first term is the steady growth of a flat surface, and the second the slowing down due to the AFP's.

The evolution of  $n$  can be calculated by noting that its rate of increase is  $k_+$ , and that its rate of decrease is the rate that AFP's are engulfed. Equation (10) implies  $\dot{q}_i = V/\log(\pi n b^2)$ . Since the  $q_i$  are uniformly distributed we must have  $dn/n = -\dot{q}_i dt/(\chi b/2)$ . This gives

$$\dot{n} = k_+ - \frac{2nV}{\chi b \log(1/\pi n b^2)}. \quad (12)$$

Eqs. (11), (12) determine the dynamics of the interface.

In the steady state we put  $\dot{n} = 0$  in Eq. (12), and use the expression for  $V$ . Thus,

$$\frac{k_+}{v_o \delta_T} = \frac{n[2 - n/n^*]}{\chi b \log(1/\pi n b^2)}, \quad (13)$$

where  $n^* = \delta_T(2\pi\chi b l_0)^{-1}$ . The right hand side of this equation has a maximum for  $n \approx n^*$ . Thus, *there is no constant speed solution* unless the right hand side is small enough, i.e., for small enough  $k_+$  or large enough  $\delta_T$ . The speed  $V$  decreases from  $v_o$  as  $\delta_T$  decreases, and, at a threshold, abruptly jumps to zero in agreement with experiment [12]. The threshold,  $\delta_T^*$  obeys

$$(\delta_T^*)^2 / \log(2\chi l_0 / b \delta_T^*) \approx (\chi b)^2 2\pi l_0 k_+ / v_o. \quad (14)$$

Eq. (14) is the central result of this work.

To test our approximations, notably the quasistatic limit for  $\phi$ , we have performed a numerical simulation of our model. The results on a  $150 \times 150$  square lattice, with each cell representing a single AFP (Fig. 2) support our analysis. The transition from the steady growth to the arrested interface regime occurs at a lower adsorption rate  $k_+$  than predicted, but the discrepancy is rather small.

Beyond the transition point, growth stops. The resulting static interface must obey Eq. (5) with  $\dot{h} = 0$ , i.e., Eq. (1). After arrest, as Eq. (12) shows,  $n$  increases as irreversible adsorption continues until limited by some aspect of surface chemistry that we have not considered.

These results hold if the region blocked by each AFP is a circle. We can also account for the fact that real AFP's are often anisotropic by assuming that the region blocked is elliptical, with semimajor and semiminor axes  $a$  and  $b$ , respectively. The potential  $h(x, y)$  can be found by conformal mapping [13]. Using this method, it is easy to show that Eq. (10) is replaced by  $H - h_i \approx -q_i / \log[\pi n(a + b)^2 / 4]$ . The slope of  $h$  will reach its critical value near  $x = \pm a$ , and its maximum will be given by the radius of curvature of the ellipse, of order

$b$ . Thus  $q_{\max} = \chi b / 2$ . With these changes, the left-hand side of Eq. (14) becomes

$$(\delta_T^*)^2 / \log[8\chi l_0 b / (a + b)^2 \delta_T^*]. \quad (15)$$

Equations (14) and (15) give  $\delta_T^*$  in terms of the geometry of the AFP ( $a$  and  $b$ ), the fundamental length  $l_0$ , and the dimensionless parameter  $\chi$ . However, Eq. (14) also involves  $k_+ / v_o$ . The rate  $k_+$  is clearly proportional to the concentration  $C$  of AFP in solution:  $k_+ = v_A C$  where  $v_A$  is a velocity.  $v_A / v_o$  is a crucial parameter.

To find this parameter in a realistic way, there is another effect which we must consider; in any experiment, soon after the adsorption process starts, water near the ice surface will be depleted of AFP. The thickness of the depletion layer depends on the diffusion coefficient  $D$ , as  $\sqrt{Dt}$ . That is,  $k_+$  becomes time dependent, and thus  $v_A \approx \sqrt{D/t}$ . The same argument applies to the rate of crystallization itself: our estimate for  $v_o$  fails as soon as the process is limited by thermal diffusion through a diffusive layer. Thus  $v_o \approx \sqrt{\kappa_T T / \Lambda t}$ , where  $\kappa_T$  is thermal conductivity of water. From the Einstein formula  $D = k_B T / 6\pi\eta b$ :

$$v_A / v_o = \sqrt{\lambda / b} \sim 10^{-2} \quad (16)$$

where  $\lambda = k_B \Lambda / 6\pi\eta\kappa_T = 1.2 \times 10^{-10}$  cm. By combining Eqs. (14) and (16) we can write

$$\delta_T^* [\log(2\chi l_0 / b \delta_T^*)]^{-1/2} \approx \chi \sqrt{2\pi b^{3/2} l_0 \lambda^{1/2} C}. \quad (17)$$

For anisotropic AFP's we must use Eq. (15). The longer axis  $a$  sets the hydrodynamic radius of the ellipsoidal particle. Its diffusion coefficient may be approximated by  $D \approx k_B T (\beta + 1) / 6\pi\eta a$ , where  $\beta = \log[(a + b) / 2b]$  (typically,  $\beta < 2$ ). Thus

$$\delta_T^* [\log(2\chi l_0 / b \delta_T^*) - 2\beta]^{-1/2} \approx \chi b \left(\frac{\lambda}{a}\right)^{1/4} \sqrt{2\pi(\beta + 1) l_0 C}. \quad (18)$$

Using these formulas, we have been able to fit the experimental data on natural AFP's of two different classes: AFP-I, and AFP-III [7,8]. They have rather different architectures: AFP-I are  $\alpha$ -helical rodlike molecules which we model as cylinders ( $b = 3.5 \text{ \AA}$ ,  $a = 25 \text{ \AA}$ ), while AFP-III have a more complicated globular structure, which we approximate as spheres ( $b = a = 8 \text{ \AA}$ ). These lengths are derived from the known structures of the molecules; the only free parameter of our theory is  $\chi$ . The theoretical curves  $\delta_T^*(C)$  are in excellent agreement with the experiments; see Fig. 3. The values of the fitting parameter ( $\chi \approx .25$  for AFP-I, and  $\chi \approx .33$  for AFP-III) are physically reasonable, close to each other, and small enough to justify the small-slope approximation used above.

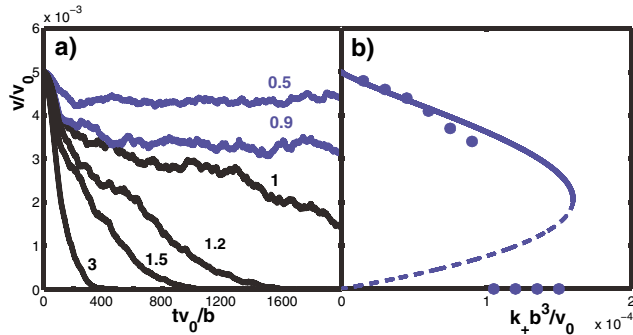


FIG. 2 (color online). (a) Simulation results for the speed of the interface as a function of time, for various values of control parameter  $k_+ b^3 / v_o$ , ranging from  $0.5 \times 10^{-4}$  to  $3 \times 10^{-4}$ .  $\delta_T = 0.005$  and  $\chi = 1/(2\pi)$ . (b) Speed in the steady state vs reduced adsorption rate: comparison of simulation (points) with analytical result (line) given by Eq. (14).

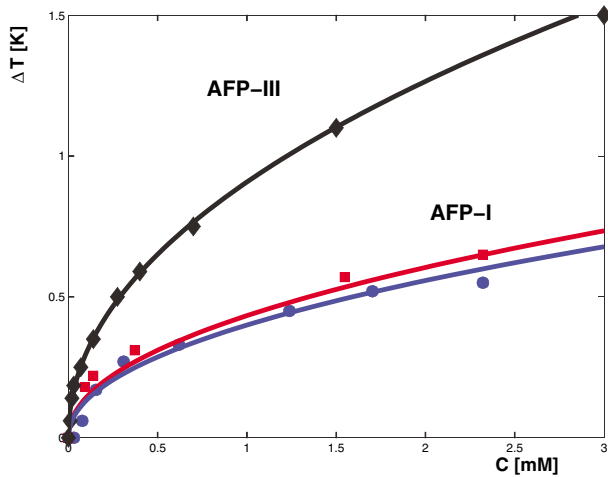


FIG. 3 (color online). Comparison of theory to experiment for maximum undercooling of water in the presence of AFP-Type III (diamonds) and two modifications of AFP-Type I (circles, squares).

According to Eq. (18), the activity is strongly dependent on the smaller dimension of the AFP. This may be useful for the design of synthetic AFP's. For example, by using ring-shaped molecules  $\delta_T^*$  could be increased since the effective size is set by the largest dimension, the radius of the ring. Our results are consistent with the measured  $\delta_T^*(C)$  for antifreeze glycoproteins. However, their molecular architecture and conformations are more complicated than those we have discussed, and their analysis would go beyond the scope of this work.

Finally, we can compare our results with the related problem of the motion of a pulled elastic interface in a medium with static obstacles to interface motion. This is of interest in the description of the kinetics of domain walls, charge density waves and flux lines in superconductors [14]. The interplay of long-range elastic coupling and local pinning results in a transition at a critical value of the pulling force, and near the transition, the average speed of the interface goes continuously to zero. Our model has pinning of the interface by the AFP molecules,

and coupling due to surface tension, but the transition is discontinuous, in agreement with experiment [12]. The difference is that the AFP's are not stationary. Thus, we call our model "kinetic pinning". There should be a crossover between the static pinning and kinetic pinning if diffusion of the obstacles is taken into account.

We would like to thank C. Knight, E. Brener, and Y. Yeh for useful conversations. L. M. S. acknowledges partial support by NSF grant No. DMS-0244419 and also the hospitality of the Kavli Institute for Theoretical Physics where this research was supported in part by NSF grant No. PHY99-07949.

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