

Dynamical mechanism of antifreeze proteins

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UND MEERESFORSCHUNG

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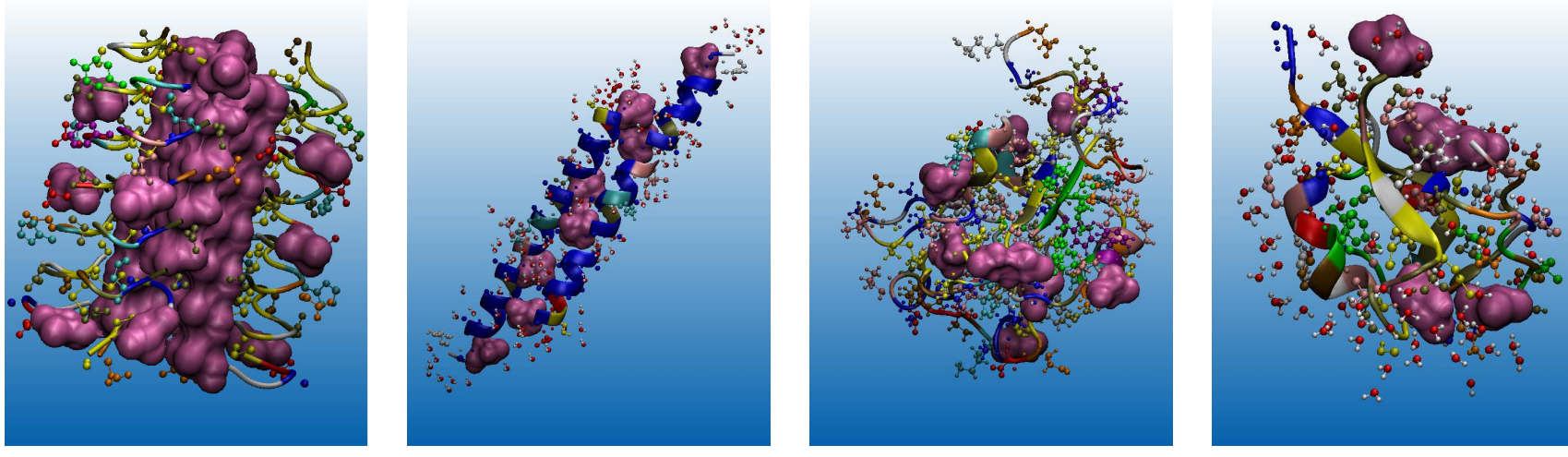
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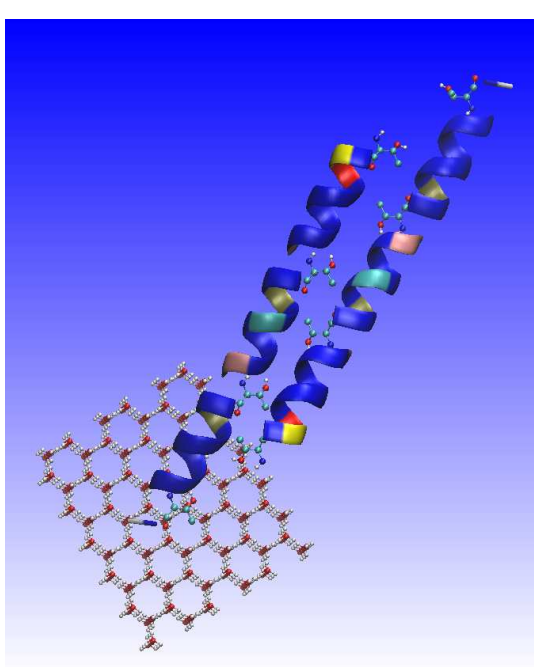
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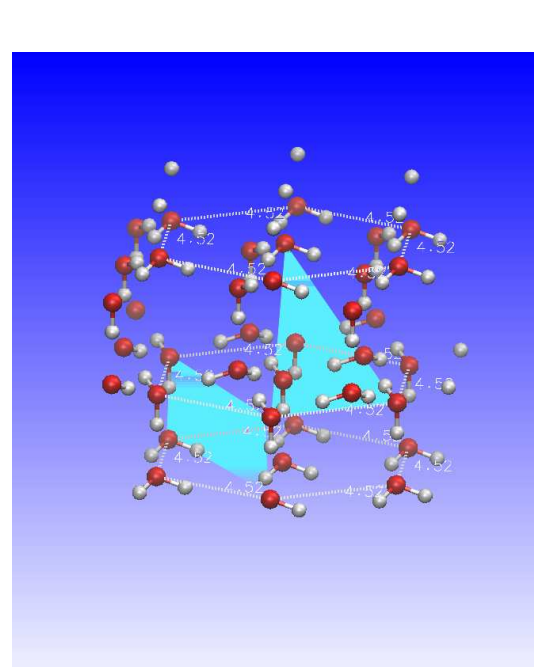
AFPs and ice-coupling



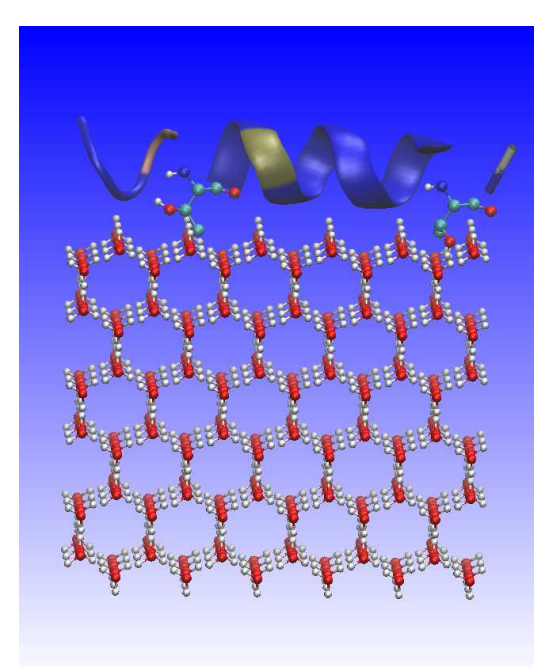
Four different classes of considered AFP structures (from left to right): tenebrio molitor (1EZG) as used in [1], psodeupleuronectes americanus (1WFB) in [2, 3], hemitripterus americanus (2AFP) in [4], and macrozoarces americanus (1MSI) in [5]. (crystallographic data in RCSB Protein Data Bank)



Alalan rich α-helical protein of the winter flounder (HPLC6)



possible adsorption planes for AFPs (type 1)



Most energetically stable binding conformation of AFP

Mechanisms ?

Colligative phenomena

Adsorption inhibition

Freezing point depression

Gibbs-Thomson (Kelvin) model

$$\Delta T = T_0 - T = \frac{k_B T_0^2}{\Delta H_0} x_b$$

$$\Delta T = T_0 - T = \frac{2\gamma T_0}{\rho_{min} \Delta H_0}$$

concentration of solvent species x_b

interfacial energy γ and inverse radius of sphere ρ_{min}

Dynamical model: two order parameters

- ice structure by **tetrahedrity** [6]
- antifreeze concentration** v

$$u \sim 1 - M_T = 1 - \frac{1}{15 \langle l^2 \rangle} \sum_{i,j} (l_i - l_j)^2,$$

l_i lengths of nearest neighbors
ideal tetrahedron: $M_T = 0$, random: $M_T = 1$

- Ginzburg-Landau-type free energy density $f(u, v) \sim \beta u + \lambda u^2 - 2\lambda u^3 + \lambda u^4 + c \left(\frac{\partial u}{\partial x} \right)^2$ allowing structures phase transition [7]
- versatile action of AFPs on grain growths simulated by activity parameter $f(u, v) \sim -a_1 uv$

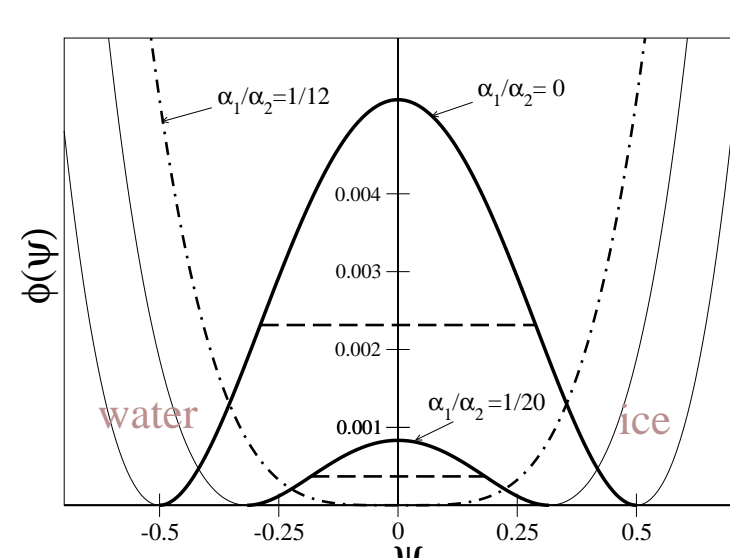
dimensionless $\tau = \frac{t}{t_0}$, $\xi = \frac{x}{x_0}$, $u = C_1(\psi + a)$, $v = C_2\rho$, with $C_1 = 1$, $C_2 = \frac{a_1}{12\lambda}$, $x_0 = \frac{1}{6} \sqrt{\frac{6c}{\lambda}}$, $t_0 = \frac{c}{72\lambda^2}$ with $\alpha_1 = \frac{a_1^2}{12^2\lambda^2}$, $\alpha_2 = \frac{a_2}{12\lambda}$ and $\alpha_3 = \frac{c\beta}{72\lambda^2}$

$$\frac{\partial \psi}{\partial \tau} = \frac{\partial^2}{\partial \xi^2} \left(\alpha_3 + \left(\frac{1}{6} - a + a^2 \right) \psi + \left(a - \frac{1}{2} \right) \psi^2 + \frac{1}{3} \psi^3 - \alpha_1 \rho - \frac{\partial^2 \psi}{\partial \xi^2} \right)$$

$$\frac{\partial \rho}{\partial \tau} = \frac{\partial^2}{\partial \xi^2} (\psi + \alpha_2 \rho)$$

- conserving **Cahn-Hilliard equation**: $\dot{u} = -\nabla j$, current itself $j \sim \nabla \Phi$, potential is variation of free energy such that $\dot{u} = -\nabla^2 \left(-\frac{\partial f}{\partial u} \right)$
- continuity equation $\frac{\partial v}{\partial \tau} + \frac{\partial j}{\partial \xi} = 0$ with AFP field v and flux $j = -\frac{\partial}{\partial \xi} (a_3 v + a_2 u)$ we get diffusion equation $\frac{\partial v}{\partial \tau} = \frac{\partial^2}{\partial \xi^2} (a_2 u + a_3 v)$ as evolution for the AFP concentration

Phase diagram by static solution $\frac{1}{2} \left(\frac{\partial \psi}{\partial \xi} \right)^2 = \Phi$

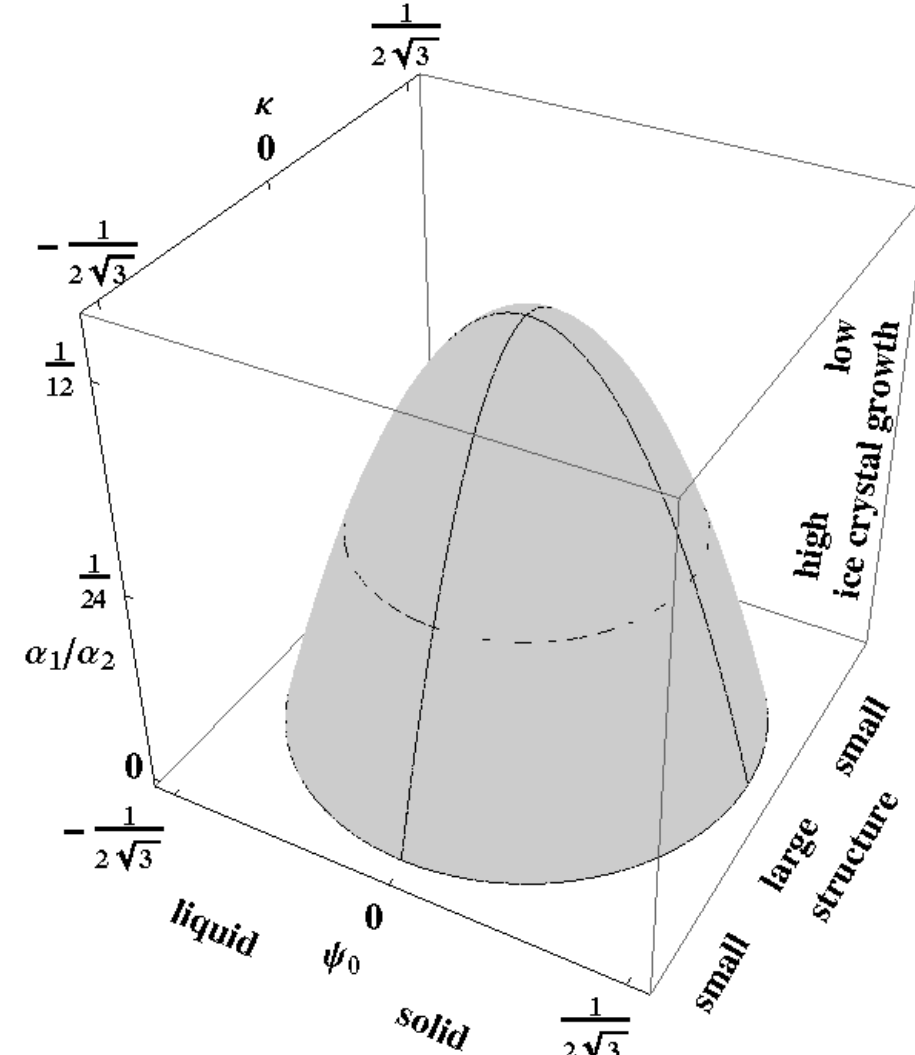


Free energy density (solid) vs order parameter for different AFP concentrations, pure ice/water $\frac{\alpha_1}{\alpha_2} = 0$ (upper line). The freezing transition interval is given by $\partial^2 \Phi / \partial \psi^2 = 0$ (dashed lines)

- Depending on α_1/α_2 and α_3 asymmetric potential Φ describing thermodynamic hysteresis, α_3 no influence on dynamics, near phase transition Φ symmetric
- symmetric potential: left and right minima: stable phase of water and ice
- concave $\partial^2 \Phi / \partial \psi^2 < 0$ region corresponds to negative diffusion coefficient leading to structure formation, flux diffuses up against concentration gradient: unstable phase transition (freezing) region reduced by AFP concentration, for $\frac{\alpha_1}{\alpha_2} = \frac{1}{12}$ double well vanishes

Linear stability analysis $\psi = \psi_0 + \psi_1 e^{\mu T + i \kappa \xi}$

- each fixed point describes a spatial homogeneous order parameter $\psi = \psi_0 = \text{const}$ and corresponds to a stationary solution of water or ice
- region of positive eigenvalues corresponds to freezing (spinodal) region
- unstable modes vanish for $\psi_0^2 > 1/12$ and also double well for $\alpha_1/\alpha_2 > 1/12$, phase transition occurs only, if fixed points are located inside of $-1/\sqrt{12} < \psi_0 < 1/\sqrt{12}$ being inside the freezing (spinodal) interval



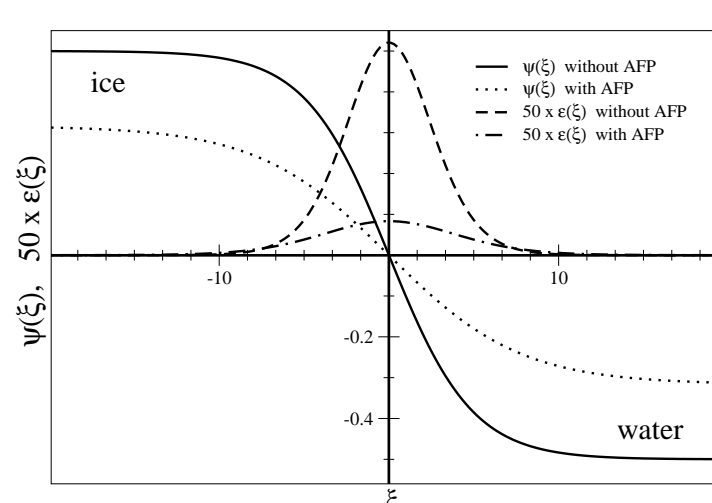
Freezing (spinodal) region dependent on the order parameter ψ_0 and the wavenumbers κ and the thermal hysteresis activity $\frac{\alpha_1}{\alpha_2}$

- size of microstructure coupled to AFP concentration and to order parameter ψ_0 which decides how much ice and water is present
- freezing region shrinks with increasing AFP concentration and vanishes at $\alpha_1/\alpha_2 = 1/12$

Surface-energy depression

- virtue of Cahn Hilliard equation is transient stationary kink solution $\psi(\xi) = -\sqrt{3N} \tanh \left[\sqrt{N/2} (\xi - \xi_0) \right]$ with $N = \frac{1}{12} - \frac{\alpha_1}{\alpha_2}$
- interfacial energy density by centered free energy $\epsilon(\xi) = f(\xi) - c = \left(\frac{\partial \psi}{\partial \xi} \right)^2$

and interfacial surface energy (tension) $\zeta = \int_{-\infty}^{\infty} \epsilon(\xi) d\xi = (2N)^{3/2}$



static kink solution and interfacial energy density with AFPs ($\frac{\alpha_1}{\alpha_2} = 0.05$) and without AFPs ($\frac{\alpha_1}{\alpha_2} = 0$)

- AFPs reduces kink between water and ice and lowers interfacial energy density (already static mechanism)
- interfacial surface energy (tension) decreases with increasing AFP coupling and vanishes at $\alpha_1/\alpha_2 = 1/12$ (limit of stability region)
- transforming back to dimensional interfacial energy: $6^{3/2} \gamma \zeta$ with our choice of surface tension $\gamma = 21.9 \frac{mJ}{m^2}$ [8], measurements provide values between $20 \frac{mJ}{m^2}$ and $46 \frac{mJ}{m^2}$ [9]
- in contrast to AFPs, γ increases linearly with salt concentration and larger critical nucleus is required to generate an interface
- salt inhibits nucleation process because of higher energy threshold whereas thermal hysteresis proteins (AFPs) reduce threshold for stable nucleus

References

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Critical cluster size

- ice crystal forms when water supercools below freezing point by growth of nucleation kernels if critical size is exceeded
- estimate of critical radius (liquid drop model): volume part of Gibb's potential $\sim -4\pi r^3 \Delta G_V / 3$, surface part $\sim 4\pi r^2 \zeta$, maximum at the critical cluster size $r^* = 2\zeta / \Delta G_V$ as long as $r < r^*$ nucleation might happen (embryo) but no cluster grows
- AFPs change interfacial energy and supercooling: change of the free energy between ice and water $\psi(\pm\infty) = \pm\sqrt{3N}$:

$$\Delta F = -2(\alpha_3 - \alpha_1 \rho) \sqrt{3N} \approx \Delta G_V$$

and critical (dimensionless) radius $r^* = \sqrt{2N}/3(\alpha_1 \rho - \alpha_3)$ decreases as the AFP concentration α_1/α_2 increases

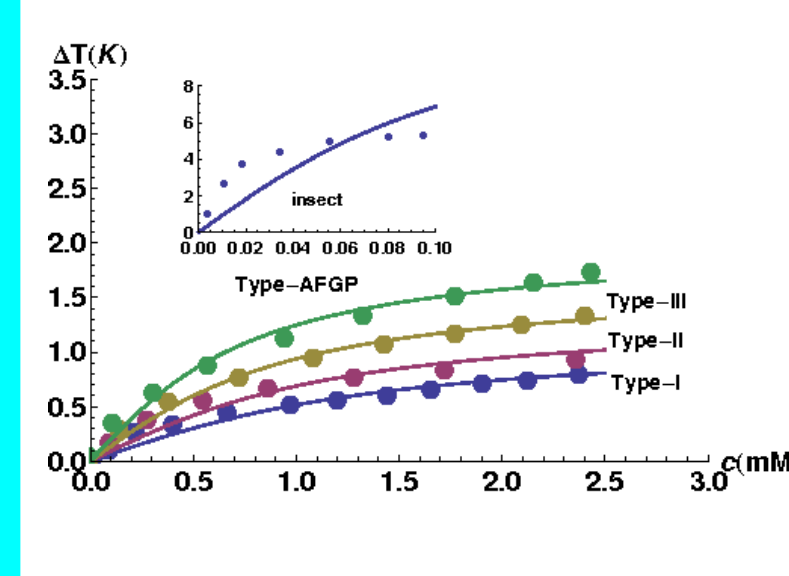
- more AFPs allow more ice nucleation but inhibit the cluster growth

Freezing-point depression: $\Delta F|_{\text{ice-water}} = \frac{\partial F}{\partial T}|_{\text{ice}} \Delta T$

- temperature dependence of AFP concentration and ice structure coupling $\alpha_1(T) = \alpha_0 + \alpha_{10}(T - T_c^0)$ with internal threshold temperature T_c^0 , activity of AFP molecules cease to act at the critical temperature $\alpha_1(T^*) = 0$, therefore freezing point depression is given by $\Delta T = T^* - T_c^0$
- with AFP-dependent supercooling temperature $T_c = T_c^0 - |\Delta T|$ $\alpha_1(T) = \alpha_0(T - T_c)/|\Delta T|$. we obtain freezing point depression or thermodynamical hysteresis observing $\psi|_{\text{ice}} = \sqrt{3N}$

$$|\Delta T| = \sqrt{\left(\frac{b}{2\rho} \right)^2 + a} - \frac{b}{2\rho}$$

with $a = 2\alpha_0(T - T_c)/\alpha_{10}$ and $b = 2\alpha_3/\alpha_{10}$

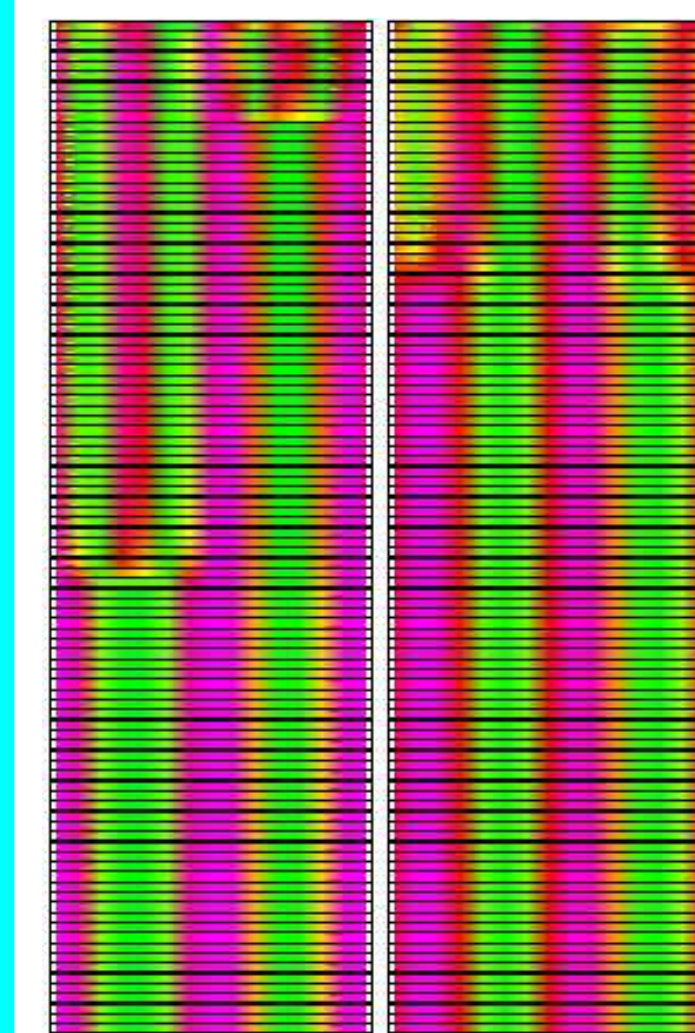


freezing temperature depression of four different classes of AFP structures [2, 4, 5] and insects [1] versus AFP concentration with fit to experimental data (points) of the collected data in [10]. AFP-specific fitting parameter:

Type	I	II	III	AFGP	insects
a	0.81	1.08	2.58	4.31	34.39
b	0.44	0.37	0.88	1.13	0.05

- nonlinear square root behavior of the freezing point depression, only for small concentrations $|\Delta T| \approx \frac{a}{b} \rho = \frac{\alpha_0}{\alpha_3} (T - T_c) \rho$ colligative freezing depression
- together with the surface tension our approach leaves one free parameter to describe further experimental constraints

Time evolution of ice growth inhibition



time evolution of order parameter versus length from 1×10^5 to 2×10^6 time steps (from above to below), left side without AFPs and right side with AFPs

- due to Cahn Hilliard equation conservation of total mass density of water but relative redistribution between water and ice
- evolution reduces number of ice grains forming a larger one after some time, accumulation occurs faster with AFPs than without, however, absolute height of ice-order parameter (ideal ice corresponds to $\Psi = 1$) is lowered by AFPs
- grain size of ice evolves faster with AFPs and remains at smaller value
- nucleation of ice starts earlier but remains locked at intermediate stage in agreement with static observation that AFPs support smaller nuclei sizes and inhibit the formation of large clusters
- this later blocking of larger cluster sizes is dynamic process due to kinetics and coupling of AFPs to the ice embryos
- width of boundary between ice and water remains larger with AFPs than without as expression of the reduction of interfacial energy

Summary

1. interaction of AFP molecules with ice crystals described by coupled phase field equations
2. two effects of AFPs: (i) interfacial energy is lowered which allows only smaller ice nuclei to be formed, (ii) ice grains are formed faster by action of AFPs but become locked at smaller sizes and smaller order parameters
3. freezing is stopped and ice-water mixture remains instead of completely freezing
4. AFPs do not prevent crystal nucleation, but inhibit further growth
5. this essentially dynamic process between AFP structure and ice-order parameter establishes a new possible mechanism for the phenomenon of anti-freeze proteins capable to reproduce the experimental data

→ B. Kutschan, K. Morawetz, S. Thoms; *Phys. Rev. E* 90 (2014) 022711