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Supplementary Information for

Ice crystal growth in the presence of a moderate ice-binding protein: Basal face growth suppression does not confer hyperactivity

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Supplementary Information Text

S1 Activity of *fc*IBP11 in pure water

In order to analyze whether *fc*IBP11 can be used in pure water, we measured the TH-activity using the nanoliter osmometer as described in Bayer-Giraldi et al. (1). Table S1 shows the data for solutions with protein concentration of 1.5 μM *fc*IBP11 and different salinities. We used protein dissolved in pure water (salinity 0 in the PSU system), in a phosphate buffered saline solution (PBS, salinity 9) and in a NaCl brine (salinity 60). We can see that the TH, defined as the difference between the freezing point and the equilibrium melting point, was not sensibly affected by salinity, although a slight increase in TH can be observed at higher salinities. This slight increase is probably due to a salting-out effect, as discussed in Bayer-Giraldi et al. (2), and we do not expect changes in the mechanisms of interaction between the *fc*IBP11 molecule and ice. Therefore, since our focus was on the physical aspect of ice crystal growth and its interaction with *fc*IBP11, we chose to work with pure water, in order to exclude cooperative effects of the protein and salts affecting crystal growth. Due to the fact that highly saline sea-ice brine corresponds to the natural environment of *fc*IBP11, we will include the aspect of salinity in our future work.

S2 Rotation of the ice crystal in the free growth cell

In the free growth cell, the rotation of the capillary allows the free rotation of ice single crystals. This gives us the possibility to observe ice single crystals from different directions. In addition to the details observed when the basal face of the crystal was parallel to the observation plane, as sown in Figure 3, we could observe the crystal with the basal face perpendicular to the observation plane. An example is shown in Figure S1. It becomes clear from these images that the crystals grew in a flat dendritic shape, with strong suppression of growth along the *c*-axis.

S3 *Relevance in sea-ice*

Considering the sea-ice context of the diatoms, its structure, and potential ecological role of the *fc*IBPs, the binding of *fc*IBP11 to the basal faces seems to be of crucial importance. Microbial assemblages are found mostly in the bottom part of sea-ice. Whereas the upper part of the sea-ice layer, in contact with the cold atmosphere, consists of isotropic granular ice crystals, the lower part gradually grows into the sea-water as parallel lamellae (3). As a result of the geometrical selection of the growing ice crystals, these grains have their *c*-axis oriented horizontally, as schematically depicted in Figure S2. Salts and other sea-water components are segregated from the growing ice lattice and accumulate within brine channels, which are elongated parallel to the lamellae and often terminate into the ocean. Brine channels constitute the living space for organisms, and most IBPs from sea-ice organisms are released from the cells into the brine where they interact with the icy walls of the channels. Looking at the structures of brine spaces, we see that the basal faces of the ice lamellae, which enclose the brine space in the vertical orientation, are the dominant crystallographic faces in the channels. Therefore, it is naturally of great advantage for *fc*IBP11 to bind to the basal face, in addition to their interaction with the other crystallographic faces. It has been shown that IBPs can influence brine retention in sea-ice (4). Brine retention over longer periods increases local ice porosity and therefore provides a living space for sea-ice microorganisms. Bayer-Giraldi et al. (2) suggested that *fc*IBP11 will accumulate in a matrix of extracellular polymeric substances and actively shape the structure of the brine space, thereby increasing its habitability. A change in ice porosity can possibly alter the geochemical imprint of sea-ice, which may be of relevance in a changing climate (5).

Fig. S1. An ice single crystal observed by bright field microscopy from different directions. The crystal was grown in the presence of 1.5 µM *fc*IBP11 and Δ*T* = 0.5°C. The image shows the crystal oriented with the basal plane parallel to the observation plane (A), with the basal plane rotate by 45° (B) and by 90° (C). Size bar = 200 µm.

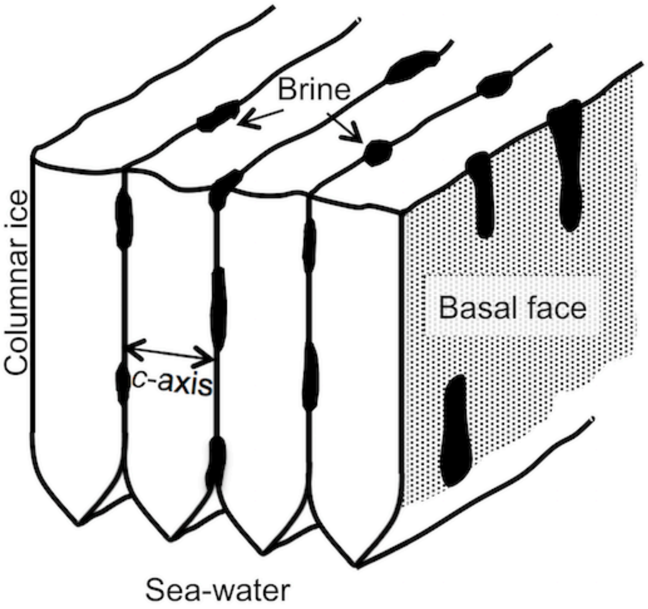

Fig. S2. Schematic drawing of the structure of columnar sea-ice. The crystals constitute parallel lamellae with horizontal *c*-axis. The brine is retained within inclusions, often elongated in the vertical direction along the basal faces of the ice crystals.

Table S1. TH of ice in 1.5 µM *fc*IBP11 solutions with different salinities.

Solution (Salinity)	TH (°C)
Pure water (0)	0.05
PBS (9)	0.06
NaCl brine (60)	0.13

Table S2. **The effect of selected moderate and hyperactive IBPs on the growth of the basal face at small supercooling temperature.**

Protein name	**Origin**	**Basal face growth suppression**	**TH activity**	**Reference**
AFP I	*Pseudopleuronectes americanus*	*no*	moderate	(6)
AFP II	*Hemitripterus americanus*	*no*	moderate	(6)
AFP III	*Anarhichas lupus*	*no*	moderate	(7)
AFP IV	*Myoxocephalus octodecimspinosis*	*no*	moderate	(8)
AFGP 7-8	*Gadus ogac*	*no*	moderate	(9)
*Mp*AFP	*Marimononas primoryensis*	*yes*	hyperactive	(10)
*Tm*AFP	*Tenebrio molitor*	yes	hyperactive	(11)
*Sbw*AFP	*Choristoneura fumiferana*	*yes*	hyperactive	(12)
*Lp*IBP	*Lolium perenne*	*Yes*	Moderate	(13)
DUF3494				
*Col*AFP	*Colwellia sp* SLW05	*yes*	hyperactive	(14)
*Efc*IBP	Antarctic bacterial metagenome	*yes*	moderate	(15)
*Fc*IBP11	*Fragilariopsis cylindrus*	*yes*	moderate	This study
*Cn*AFP	*Chaeotoceros neogracile*	*yes*	moderate	(16)
AFP1	*Glaciozyma antarctica* PI12	*yes*	moderate	(17)
*Tys*AFP8	*Typhula ishikariensis*	*yes*	hyperactive	(18)

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