Controlling ice crystal growth with ice-binding proteins

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Crystallization of water into ice is lethal to most organisms and detrimental to many soft materials. Freeze-avoiding fish living in polar seas have evolved to tackle this problem with an unusual coping strategy. They produce so-called antifreeze proteins (AFPs) that block the growth of nascent ice crystals within a narrow temperature range known as the thermal hysteresis gap enabling survival under extreme conditions [1]. Encoding this functionality into synthetic polymers would open up new avenues for e.g. cryopreservation, de-icing technologies and advanced coatings.

We study how and why ice-binding proteins (IBPs) bind onto specific ice crystal planes and its impact on the various functional roles of ice-binding proteins (freezing point depression, inhibition of recrystallization, crystal shaping, etc.) using a range of activity assays [1, 2]. We aim to achieve a solid mechanistic understanding of how IBPs work as a crucial first step for the knowledge-based design of potent synthetic ice crystal growth modifiers.

An evaluation of thermal hysteresis (TH) and ice recrystallization inhibition (IRI) activity of all major classes of AFPs using cryoscopy, sonocrystallization, and recrystallization assays reveals a marked difference in TH activities determined by cryoscopy and sonocrystallization, while TH and IRI activities are not correlated [2]. This points to a mechanistic difference in ice growth inhibition by various types of AFPs: basal plane adsorption is relevant only at long annealing times and at small undercooling, while blocking fast ice growth requires rapid adsorptions on other crystal planes. Interestingly, an ice-like vibrational signature is observed in the sum frequency generation (SFG) spectrum taken at room temperature of the only antifreeze protein that displays virtually the same TH activity (Figure 1) in cryoscopy (slow growth) and sonocrystallization (fast growth), suggesting that ice-binding of this protein (rQAE) is mediated by its hydration shell [3]. The characteristic ice-like feature disappears upon a single point mutation in the ice-binding site from threonine to asparagine, which also eliminates activity. These findings shed light on the working mechanism of IBPs and offer direction to design synthetic macromolecular antifreezes [4].

[1] A. S. Oude Vrielink, A. Aloi, L. L. C. Olijve, I. K. Voets *Biointerphases*, 2016, **11**, 018906.
[2] L. L. C. Olijve, K. Meister, A. L. DeVries, J. G. Duman, G. Shuaiqi, H. J. Bakker, I. K. Voets *Proc. Natl. Acad. Sci.* 2016, **113**, 3740.
[3] K. Meister, S. Strazdaite, A. L. DeVries, S. Lotze, L. L. C. Olijve, I. K. Voets, H. J. Bakker *Proc. Natl. Acad. Sci.* 2014, **111**, 17732.
[4] L. L. C. Olijve, M. M. R. M. Hendrix, I. K. Voets *Macromolecular Chemistry and Physics*, 2016, in press.