Dynamic measurement of ice growth by atomic force microscopy in aqueous solutions in the presence of ice-binding proteins

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Besides enabling organisms to thrive in cold environments, ice-binding proteins (IBPs) hold great promise in tissue preservation and food processing. These proteins lower the freezing point by binding to ice surfaces, thus inhibiting crystal growth. Despite their importance, the microscopic action of these proteins is largely unknown due to until-now unresolved difficulties in achieving high-resolution, in-situ imaging. We present a novel system design that enables dynamic atomic force microscopy (AFM) imaging of the ice-IBP system. We studied two different types of protein systems, one exhibiting moderate thermal hysteresis and the other hyperactive. These proteins bind to different faces of the growing ice crystals, leading to characteristic structures rationalized by selective inhibition. AFM images reveal such structures at the tens of nm scale for the first time and will be discussed in relation to previous, lower-resolution optical images.

Several challenges had to be overcome to achieve these results, including moderating and controlling heating from the AFM head and detection laser, preventing cantilever freezing and isolating, and monitoring the boundary between liquid and solid as observed at the growing ice front. Using the new set-up enabled control of ice growth in the presence of IBPs, and even demonstrated the feasibility of measuring less-controlled ice growth in the absence of the IBPs. Small pits were observed near the apex of growing tapered structures, which can be understood in light of the attachment of the IBP to specific planes. Although several studies on crystals growing in thin films exist, this is the first demonstrated imaging of a growing bulk crystal immersed in its own melt with AFM. This study suggests the feasibility of imaging and measuring the mechanical properties of surface adhered cells in a partially frozen environment, opening new avenues in cryobiology.