# Fish ice-binding proteins differently function at higher concentrations

**Sakae Tsuda[1], Tatsuya Arai[1], Kazuhiro Mio[2], and Yuji C. Sasaki[1,2]**

[1] Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa 277-8561, Japan.
[2] AIST-UTokyo Advanced Operando Measurement Technology Open Innovation Laboratory, National Institute of Advanced Industrial Science and Technology, Kashiwa 277-0882, Japan.

It has been found 4 types of fish ice-binding proteins (IBPs), which are denoted AFP I−III and AFGP. Their molecular functions, such as the thermal hysteresis (TH) activity, have generally been examined up to the concentration of approx. 10 mg/ml, which evoked us a question about their concentration-dependence up to the solubility limit. Here we prepared natural samples of AFP I−III and AFGP to examine their water solubility, thermal hysteresis activity (TH), ice-shaping ability, and fluorescence-based ice plane affinity (FIPA) at various concentrations. It appeared that they are extremely soluble in 4°C-chilled water, such as 1,800 mg/ml (AFGP), 400 mg/ml (AFP I), 200 mg/ml (AFP II), and 100 mg/ml (AFP III). It appeared that their maximal TH values were 2−3°C (at 50−200 mg/ml), which are higher than the reported values. For AFP I, the bursting ice crystal growth toward *a*-axes was detected at the concentration of 150 mg/ml, while it exhibited an ordinary needle-like growth toward *c*-axis at 5 mg/ml. The FIPA analysis showed that (1) AFP I binds to the pyramidal planes of a single ice crystal at lower concentrations (0.01 mg/ml), while it extends the target area to whole ice crystal planes at higher concentrations (0.1 mg/ml), (2) AFP II also extends the target area from 2ndary prism planes to whole ice crystal planes at higher concentrations, (3) AFP III binds to only 1st prism- and pyramidal planes and does not extend the target area at higher concentrations, and (4) AFGP binds to only 1st prism plane and does not extend the target area at higher concentrations. These results reveal that fish AFPs behave differently at higher concentrations, which might be ascribed to a difference of their structural and hydration properties on the ice crystal surfaces [Refs].

Sakae Tsuda: Research fellow, Graduate School of Frontier Sciences, The University of Tokyo.

References: Biomolecules (2020), 10, 423; SciRep (2019) 9, 2212; PNAS (2018) 115, 5456.

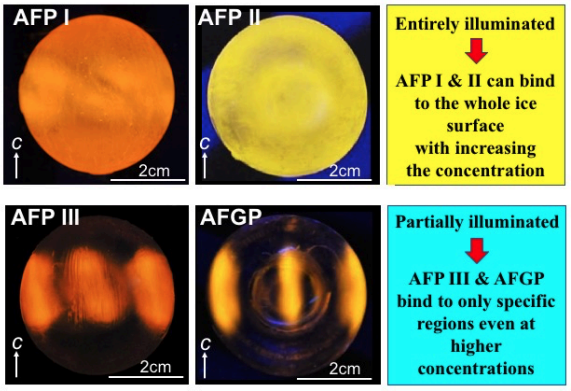


Figure 1. FIPA patterns observed for AFPI-III and AFGP. At a lower concentration (0.01 mg/ml), AFP I and II illuminate limited regions similarly to AFP II and AFGP.