

## Poster Presentation

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### *Structure-based classification of ice-binding proteins*

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Since the antifreeze protein (AFP) super family has low structural identity, classification standard of the AFPs is presently ambiguous. Newly identified ice-binding proteins (IBPs), named so after the function of the AFPs, have similar structural identity and function that interact to the ice. Identification and characterization of IBPs from the eukaryotic microorganisms *Typhulaishikariensis* (TisAFP) and *Leucosporidium* sp. (LeIBP) revealed that both are glycosylated and have irregular motif on the ice-binding site (IBS). The IBPs share a unique right-handed  $\beta$ -helix, which provides an advantage of broad-range interaction surface. The other IBP encoded by the Antarctic bacterium *Flavobacterium frigidis* PSI was determined at 2.1-Å resolution to gain insight into its ice-binding mechanism. The structure of FfIBP shows the presence of an intra-molecular disulfide bond in the loop region between  $\alpha$ 2 and  $\alpha$ 4 (capping region), unlike that of LeIBP and TisAFP. Electron density for this disulfide bond was seen between Cys107 and Cys124 during the structure refinement process and the C $\beta$ -C $\beta$  distance between Cys107 and Cys124 was 3.9 Å. By sequence alignments and structural comparisons of IBPs, we defined two groups within IBPs, depending on the sequence differences between the  $\alpha$ 2 and  $\alpha$ 4 loop regions and the presence of the disulfide bond. In addition, to investigate the effects of the capping region on the activity and stability of FfIBP, we determined the crystal structure and measured the thermal stability of mutants that swapped the capping region of FfIBP and LeIBP (mFfIBP and mLeIBP). In thermal denaturation experiments, it is clear that the capping-head region of FfIBP is more stable than that of LeIBP and is important for the overall stability of IBP, although it is not directly involved in the antifreeze activity.

[1] Do, H., Lee, J. H., Lee, S. G. & Kim, H. J. *Acta Crystallographica Section F: Structural Biology and Crystallization Communications* (2012), 8, 806-809.

[2] Lee, J. H., Park, A. K., Do, H., Park, K. S., Moh, S. H., Chi, Y. M. & Kim, H. J. *Journal of Biological Chemistry* (2012), 87, 11460-11468.

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