

**Does the antifreeze protein from the bacterium, *Marinomonas primoryensis*, function in binding ice or stopping ice from growing?**

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A  $\text{Ca}^{2+}$ -dependent antifreeze protein was isolated from the Antarctic bacterium *Marinomonas primoryensis* (*MpAFP*) found in Ace Lake. *MpAFP* is an exceptionally large multi-domain protein (ca. 1.5 MDa) that is divided into five distinct regions by two highly repetitive domains, region II (RII) and region (RIV). *MpAFP\_RIV* is the only part that has antifreeze activity and its beta-helical structure has been recently solved by X-ray crystallography. However, the 322-aa *MpAFP\_RIV* only makes up 2% of this massive protein, and therefore the antifreeze activity produced by RIV is not likely to be the sole function of *MpAFP*. In contrast, *MpAFP\_RII* consists of 120 identical 104-aa repeats, and accounts for over 90% of *MpAFP*. CD analysis has shown that *MpAFP\_RII* adopts a structure that is rich in beta-strands in the presence of  $\text{Ca}^{2+}$  and is largely unstructured in its absence. Moreover, bioinformatics analysis of RII indicates a relationship to outer membrane adhesin proteins. If *MpAFP* is located at the outer membrane of the cells, RIV could potentially function, not as an antifreeze protein, but to bind the bacterium to ice. An affinity for ice could potentially help localize *Marinomonas primoryensis* near the water surface where it might have better access to oxygen and nutrients.

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