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Investigating the higher order structure of a bacterial ice nucleation protein

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Bacterial ice nucleation proteins (INPs) contain a previously unnoticed domain: a set of arginine-containing coils at the C-terminal end of the central solenoid. This central solenoid section comprises ~50-80 sixteen-residue tandem repeats each of which is a coil of the solenoid. The N-terminal ~80% of these coils have arrays of putative water-organizing motifs like those seen in insect antifreeze proteins but on a much larger scale. Following these water-organizing (WO) coils are 10-12 repeats that follow a different consensus sequence. They lack the water-organizing motifs and the stacked tyrosines. On the opposite face of the solenoid to the missing tyrosines, the high occupancy by negatively charged residues (aspartic/glutamic acid) is switched to positively charged residues (mainly arginine), which led us to call these repeats R-coils. Using a series of mutations, deletions, and rearrangements, we have shown that the quantity, charge, and C-terminal location of these R-coils are all critical for ice nucleation activity. We hypothesize that the R-coils are required for oligomerization of INPs into large aggregates, which is required for ice nucleation activity at high sub-zero temperatures, and suggest a model for this oligomerization.

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