

“Tetramer is the minimum size of the INP aggregate in *P. Syringae*”

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Abstract: Ice nucleators of biological and abiotic origins present in aerosols are responsible for facilitating the heterogeneous freezing of atmospheric water droplets at temperatures above -35°C. Ice-nucleating proteins (INPs) of the plant-associated bacteria *P. Syringae* are among the most efficient ice nucleating materials, promoting crystallization at temperatures as high as -2°C. Apart from the structural properties of the ice nucleation sites, the exceptional activity of bacterial ice nucleators has been reported to depend on aggregation of INPs.^[1-4] The central enigma of bacterial ice nucleation arises from the existence of two preferred ranges of ice nucleation temperatures denoted as class A and class C. Determining the size of the aggregates responsible for these two classes is key to understand their working mechanism. Here we combine experimental data, stochastic optimization, and classical nucleation theory (CNT) to show that monomers and dimers are not present in the membrane of *P. Syringae*. By extracting the underlying distribution of freezing temperatures from the cumulative freezing spectrum (N_m), we decompose the total population of ice nucleators into subpopulations.⁵ Combining this analysis with CNT predictions,¹ we conclude that the ice nucleation in class C is promoted by tetramers, while nucleation in class A is due to higher order oligomers.

Acknowledgements: We gratefully acknowledge support by AFOSR through MURI Award No. FA9550-20-1-0351.

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