

## ***Interactive comment on “Freezing nucleation apparatus puts new slant on study of biological ice nucleators in precipitation” by E. Stopelli et al.***

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The method described in this paper for conserving samples for freezing nucleation studies over long periods of time, with or without deliberate modifications, opens new possibilities for addressing the difficult problem of clarifying some of the factors that govern heterogeneous ice nucleation. Dorsey (1948) made effective use of this method with a total of 84 samples contained in glass bulbs and over periods of up to two years. Each sample was tested individually each time. To my knowledge, only sporadic attempts were made in the intervening half a century to follow that arduous path.

The results presented in Section 3.1 demonstrate an active change in the sample, i.e. an evolution. The heading “effect of storage” doesn’t do justice to that. On the other

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hand, the interpretation offered for those changes requires more proof. For example, what factors may influence the aggregation versus disintegration of protein structures? Random effects? Some size threshold? In a broader sense, are there other possible explanations for the observations? Removal of some interferences on the nucleating sites? Generation of new sites?

In Fig. 4, it would be useful to be able to trace which samples move up to higher temperatures. This could be done with some color coding of the samples that appear in the higher group with a corresponding color in the previous panel. Did any of the samples from the -4 to -5 group move to colder temperatures?

The dilution tests are roughly consistent with the idea that the amount of bulk water surrounding an ice nucleating particle (cell or fragment or else) doesn’t effect significantly the temperature of activity. The data shown cannot be said to be conclusive because there are decreases in the freezing temperatures of even the best samples of each sequence. The loss of activity after two dilutions in two of the samples and in two more after three dilutions is troubling. It isn’t easy to think of explanations for these losses and the authors didn’t offer one. Still, it appears attractive to pursue this approach as a method of purification/isolation of the most active nucleating particles for complementary analyses.

Reference: Dorsey, N. E. 1948: The freezing of supercooled water. *Trans. Amer. Phil. Soc.*, 38, 24-328

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