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Atmospheric Chemistry and Physics Discussions

Interactive comment on "Protein aggregates nucleate ice: the example of apoferritin" *by* María Cascajo-Castresana et al.

Anonymous Referee #2

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The authors describe the ice nucleation active entities of biological materials and highlight the ice nucleation activity of proteins and viruses. The bulk freezing experiment DRINCZ is used to investigate 96 wells at the same time. Common proteins were screened; a particular focus was on ferritin in its iron-containing and iron-free modification. The authors conclude that ice nucleation activity seems to be a common feature of diverse proteins, irrespective of their function, but arising only rarely, most probably through defective folding or aggregation to structures that are ice nucleation active

This paper is well-written and the topic fits into the journal Atmospheric Chemistry and Physics. The paper should be published after some changes, which are listed below:

1. Thoroughly describe the basic principles of proteinaceous ice nucleation in the introduction. How did other authors describe the correlation between the sizes of the

proteins/aggregates and their ice nucleation activity? What are the differences between free proteins and those embedded in the outer membrane? Quote Pummer et al. 2015 and literature quoted within.

2. When mentioning the ice nucleation activity of Pseudomonas syringae, you might also explain the aging of P. syringae, which drops the freezing temperature by more than 5°C only due to storage in the dark at temperatures below 0°C (see e.g. Häusler et al. 2018). What are the reasons for the aging effect? Changing of size can be excluded at these conditions. How does this effect correlate to your findings?

3. You might consider that aggregation is important not only between proteins but also between proteins and polysaccharides (e.g. cellulose). Please quote Felgitsch et al. 2018 and literature quoted within.

4. P. syringae has large ice-templating sites, which most other proteins do not exhibit. Aggregation and deective folding will not generate such ice-templating sites. What kind of ice nucleation mechanism do you anticipate for the proteins in your study?

References

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