

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

**Anonymous Referee #1**

Received and published: 17 December 2019

This manuscript describes observations of high temperature ice nucleation activity of some common proteins and a virus. The introduction and discussion point toward their relevance as potential cloud aerosols. There is, however, very little background on the properties of proteins/compounds that serve as ice nucleators. The efficacy of an aerosol to serve as an INP largely depends on its chemical/mineral makeup, morphology, and size. None of these properties are introduced and sufficiently discussed. In addition, the rationale for the experimental design and selection of these specific proteins for analysis is not presented.

I believe the results presented are interesting in that they provide insight into the (unexpected) types of proteinaceous compounds that can serve as efficient INPs. However, there are a number of items in the presentation that must be clarified before this article

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should be considered for publication.

Specific comments:

Pg. 2, Lines 23-25: The first two sentences give the impression of a discussion on marine INPs but are unrelated to the content that follows in this paragraph. Please considering editing for clarity.

Pg. 2, Line 30 and throughout manuscript: Please do not capitalized the species name, i.e, *Pseudomonas syringae*.

Pg. 2 Line 33: Suggest replacing “populate surfaces” with “leaf surfaces” or describing the bacteria as epiphytes.

Pg. 3, Line 4: “Here we focus on proteins, which are the most or second-most important biological INPs.” Please explain the basis for this statement. Presumably it is in reference to an atmospheric context.

Pg. 5, Line 35: Please explain what this means (skimming David et al. 2019 didn't help easily figure this out).

Pg. 6, Lines 30-31: This step would create water loss due to evaporation during the treatment. Was the protein concentration determined after the treatment or was concentration corrected for the evaporated volume?

Pg. 7, Lines 21-23: Please explain the rationale for the protein concentrations used in the experiments. For example, why were all not tested at the same molar concentration?

Pg. 8, Lines 12-13: Please explain the basis of this conclusion. Is there evidence from other measurements that the quaternary structure of the protein was disrupted in batch 2?

Pg. 8, Lines 19-21: Unclear how the presence/absence of Fe is inferred. Would dialysis be expected to remove Fe bound to the ferritin protein?

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Pg. 13, Lines 13-14: First, the IN protein is in the outer membrane of these bacteria, not their cell membrane. Second, I'm not certain this comparison is a good one since heat-treated IN proteins of *P. syringae* lose their activity.

Pg. 14, Lines 13-14: The statement that cells must be disrupted to display IN activity is not accurate (e.g., Christner et al. 2008, 319:1214).

Pg. 14, Lines 22-23: I had trouble following this argument. Are you referring to aggregation in the wet phase? If not, how do dry aerosols become diluted?

Figure 2: What are the dotted lines co-plotted? Confidence intervals?

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Interactive comment on Atmos. Chem. Phys. Discuss., <https://doi.org/10.5194/acp-2019-861>, 2019.

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