

Interactive comment on “Freezing from the inside. Ice nucleation in *Escherichia coli* and *Escherichia coli* ghosts by inner membrane bound ice nucleation protein InaZ” by Johannes Kassmannhuber et al.

Anonymous Referee #1

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Kassmannhuber et al. present an interesting study concerning the ice nucleation activity of a protein from *Pseudomonas syringae* transferred to the inner membrane of *Escherichia coli* ghost shells. The paper is well-written and the scientific content is sound, but the structure of the paper doesn't follow the rules of ACPD and is rather confusing. The main problem, however, is that the content is not related to atmospheric chemistry and physics. Of course, one could easily find atmospheric implications in climate geo-engineering or in artificial snowing, but the authors do not even try to find a connection with the atmosphere. Therefore, I have doubts if ACP is the appropriate journal for

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this research and if not a biological journal would fit much better. If the authors and the editor should decide to stay with ACP, then major changes of the manuscript are needed.

In the introduction an additional chapter is necessary, where the authors should describe the connection between biosphere and atmosphere including transport processes and what is known about bacteria in the atmosphere. They should explain how bacteria are nucleating ice, where bacterial habitats are situated and why bacteria have developed the property of ice nucleation. Also the atmospheric ice nucleation mechanisms and the protein chemistry should be explained in more detail.

The 4th chapter “materials and methods” is very fragmentary. Important information about the set-up and the evaluation of the nucleation data are missing. The authors quote Kassmannhuber et al 2017 concerning the set-up, but when reading this paper I couldn't find the crucial information concerning the set-up. All I learned was that the droplets are pretty large about 1mm³ in volume, which makes the data incomparable with other publications in the field. Also the set-up has never been compared with other set-ups using standard samples (see. e.g. Häusler et al. 2018 or Harrison et al. 2018). I also miss the cooling rates of the experiments, which has an important impact on the results. A reference concerning the SFG measurements would help to understand the spectroscopic set-up. (p.8, l.37) For the data evaluation the authors refer to Vali 1971. However, only recently Vali 2018 has been published in AMTD revisiting the former methods from 1971. The authors should update their methods accordingly and should replot their data as $K(T)$. Eventually also ns data should be calculated for comparison.

The 2nd chapter “Results” is very much into biochemistry and are difficult to read for meteorologists, and atmospheric physicists and chemists. Figure 3 includes too less data points, which makes the T50 values very imprecise. The cooling rate would help to understand this data. The 3rd chapter “Discussion” is short and atmospheric implications are missing. A conclusion chapter has not been presented.

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Minor revisions

Tables should be formatted according to the guidelines Abbreviations should be controlled for double meaning. Atmospheric scientists use e.g. INP as ice nucleating particles, INM is ice nucleating macromolecule, INA is ice nucleating activity etc.

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