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Atmospheric Chemistry and Physics Discussions

# *Interactive comment on* "Immersion freezing of ice nucleating active protein complexes" *by* S. Hartmann et al.

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The authors thank Anonymous Referee #1 for the helpful comments. Considering the referee's opinions we restructured and rewrote more or less the entire manuscript. Especially the "introduction" and "summary and conclusion" sections have been significantly modified. This makes it sometimes impossible to relate specific referee comments to specific changes in the text. We apologize for the resulting more general references to certain sections of the manuscript.

In the following the referee's comments are in italic and the corresponding answers in regular letters.



MAJOR COMMENTS referee # 1:

*Hartmann et al. use the now well known and highly capable LACIS flow tube to study the ice nucleation efficiency of SNOMAX, a commercial ice nucleating bacteria used in e.g. artificial snow production. The effect of bio-aerosol on ice nucleation has been the focus of many recent laboratory studies and SNOMAX has now been well characterized by a number of groups; previous work is well referenced here.*

*The novelty of this manuscript is therefore not in the freezing behavior of SNOMAX - which would not, by itself, warrant another publication in a journal such as ACP since it has been done frequently before - but instead is to place the results within a framework that tries to understand freezing based on the number of (ice nucleation active) "complexes" present within droplets. Unfortunately the physical nature of what a complex is isn't fully developed in this manuscript, nor is if complexes are units of bacteria inherent to a commercial product (which SNOWMAX is), a by-product of laboratory generation, or as the authors appear to assume a parameter relevant to the atmosphere. This, more than anything, must be corrected before possible publication.*

In the new version of the manuscript, and here especially in the "introduction", which has been restructured and rewritten as a whole, the concept of the INA protein complex is in our opinion now far better developed and defined. It is also shown in the manuscript, that the number of ice inducing entities in a droplet influences the freezing temperature of the droplet (particularly in section 3.4) and it is discussed that hence onset temperatures may not be the proper parameter for quantifying the ice nucleation behavior. Therefore, our study is not just a repetition of results which existed before, but instead derives an ice nucleation rate for single protein complexes which provides a far more general base for modeling immersion freezing by bacterial cells than existed before.

Furthermore, some suggestions on how to implement the paper's findings into atmospheric models are given in the "summary and conclusions" section.

*That being said, this paper may be publishable in ACP after major revisions. As stated, the definition of complex remains undefined and certainly is not a value that has ever been measured in the atmosphere. My first suggestion is to provide an exact meaning of what a "complex" is : in the manuscript I find assertions that it is bacterial fragments over* $100$ *nm in size, it is a portion of a cell wall that promotes ice formation, and it is confused with the onset temperature of ice nucleation of different proteins (so-called types A, B, C of Turner et al. 1990).*

Again we refer to the new "introduction", and also to section 3.1, where we explain that the INA protein complex we are looking at is an aggregate of two up to a few ice nucleating proteins implemented in outer cell membrane of the bacterial cell wall (being identical to the class III found in INA bacterial cells as described e.g. by Yankofsky et al. (1981) and Turner et al. (1991)).

*The reader is currently unable to interpret if a "complex" is related to the production method of SNOWMAX (i.e., is it a bacterial fragment of a certain size related to the production method grinding - to maximize ice nucleation?) or is it a result of the preparation of the bacteria in the lab (a by-product of the droplet production method)?*

The INA protein complexes we are looking at are the smallest ice nucleating entity which *Pseudomonas syringae* bacteria form. As said above, an INA protein complex is now better defined in the "introduction", and a comparison to literature data (section 3.4) shows that those complexes we are looking at are not only valid for Snomax$^{TM}$, but also can describe freezing behavior of *Pseudomonas syringae* bacteria. The



complexes are independent of the production method of Snomax$^{TM}$ and also of the particle generation (there is a new section now on the generation of the particles, section 2.1). Only the abundance of the complexes in a Snomax$^{TM}$-batch might be batch-dependent, which, however, would not influence our results, as we derived the ice nucleation rate for a single complex by accounting for the average number of INA protein complexes being present in the sample.

*How is the abundance of complexes actually relates to atmospheric biological material? This will need to be discussed at length in revision.*

This question is difficult to answer at the current stage, as the number of INA protein complexes per cell is still not well constrained (most likely it's one or zero) and possible enrichment effects, e.g., in soils, are not well understood and quantified. However, if INA protein complexes can occur separate from bacteria, and in our view there is no reason why they shouldn't (see e.g. Kleber et al. (2007) and Conen et al. (2011) now included in our manuscript), we need to quantify both, their abundance in the atmosphere (needs to be done), and their nucleation behavior (dealt with in this paper).

The "introduction" now gives more information about what is known with respect to bacterial abundance in the atmosphere to date.

So altogether, again we refer to the highly modified "introduction" and "summary and conclusion" sections.

*I further note that there are several statements made about differences between this and the work of Moehler and agreement with Wood. I wonder and would like the authors to comment if this might be due to different samples of SNOWMAX with different properties.*

Möhler et al. (2008) assumed only particles in the size larger $600$ nm to be ice nucleation active. We show, that also particles at smaller sizes can nucleate ice. In fact, the ice fraction found in our polydisperse experiment ($0.0055$) is in the same order of magnitude as the one that could be estimated from Möhler et al. (2008) data ($0.009$) when taking into account that also particles below $600$ nm are ice active. This, in our view, indicates that the differences are not mainly caused by differences in Snomax$^{TM}$ properties, but rather due to ice nucleation being induced also by particles of smaller sizes. However, as considered temperatures were not identical and particle size distributions may have been different, despite the similar techniques used for particle generation, we prefer to only state the main difference between Möhler et al. (2008) and this work, i.e., that particles smaller than $600$ nm can be ice active.

The respective paragraphs have been re-written and the data from Möhler et al. (2008) has been removed from section 3.4.

*For example, what if SNOMAX is sometimes ground to one size distribution and sometimes another? Can this be precluded? My suggestion here is for some off-line size distribution work to be done along with some contact with the company to provide data.*

The advantage of our approach, i.e., first determining the average number of INA protein complexes per particle/droplet, and based on this, the ice nucleation rate of the INA protein complex, the results are independent of the initial Snomax$^{TM}$ size distribution. More details are given below.

*Also, the authors try to extrapolate their freezing model "CHESS" to atmospheric cloud modeling (statements are made in the abstract and summary). Many critical steps are missing between the lab model and cloud, however, and these are never actually discussed. As it stands the statements seem to be used to increase the importance of*



*this model by suggesting − but never supporting − this assertion. The "missing steps" include the abundance of bacteria in the atmosphere, the fraction that are ice active, and how the "complexes" discussed in the paper related to e.g. bacterial fragments in the free atmosphere. If the authors want to maintain this possible link from CHESS to cloud parameterizations these intermediate steps must be addressed in the revised manuscript; it is not sufficient to simply suggest there may be a possible use here.*

This is strongly related to the above question how the abundance of INA protein complexes relates to atmospheric biological material. The main issue in this context is the number of protein complexes being present in atmospheric aerosols, a property that needs to be constraint by atmospheric measurements. With this parameter being known, CHESS model and nucleation rate could be used for atmospheric modeling purposes as suggested and briefly outlined in the new "summary and conclusion" section.

*Otherwise, I find this a well written paper with reasonable length and high quality figures. Since much of the data is already found in the literature the aforementioned points absolutely must be addressed if this is to be ultimately published in ACP otherwise this paper is a repeat of previous measurements, albeit with a new chamber.*

*Some specifics on major point 1: e.g. Abstract (Line 10): "The experiments performed in the lower temperature range, where all droplets freeze which contain at least one INA protein COMPLEX, are used to determine the average number of INA protein complexes present, assuming that the INA protein complexes are Poisson distributed over the droplet ensemble." (complex is my highlight) Surprisingly, the term "single complexes" is never fully defined nor is how they relate the atmosphere? It seems to me that these "complexes" are actually the smallest unit of bacteria IN THESE STUDIES which may not have a bearing on the atmosphere (that is to say the authors*

*haven't addressed what a complex is, how it is produced, what its physical properties are). Relation to other studies likely means they are related SNOMAX properties, or perhaps lab conditions, not atmospheric relevance. To further this point the figures and text appears to indicate a complex is* $100$ *nm in size (i.e., particles smaller than this are devoid of ice nucleation). This is certainly not the size of a protein nor is it the size of a bacterium. It might be the size of some features on cells which promote ice nucleation but this is never defined. What is it then, a fragment of a bacterium? What causes such fragments in SNOWMAX? Is it industrial preparation to maximize ice nucleation efficiency? Is this related to actual bacterial fragmentation in the atmosphere or a byproduct of industry? Are such fragments always* $100$ *nm in size or sometimes different depending on preparation? If the later then this is NOT how one would want to parameterize atmospheric ice nucleating bacteria. Central point: why should we consider "complexes" representative of the atmosphere and not simply this experiment?*

We refer to the newly rewritten "introduction", section 2.1 and "summary and conclusion" sections. The abstract has been re-written as well.

*More on major point 2: e.g. Abstract (20): "The results obtained in this study allow a new perspective on the interpretation of immersion freezing experiments considering INA protein complexes and the derived simple parameterization of the heterogeneous ice nucleation rate can be used in cloud resolving models for studying the effect of bacteria induced ice nucleation." (this is repeated in summary). This is a huge leap from lab to atmosphere. Indeed, the authors don't demonstrate how the lab studies connect to the atmosphere but then produce a model parameterization. There are several steps between that must be first considered: (1) how common are bacteria in the atmosphere? (2) of these how frequent are IN-active species such as SNOWMAX? (3) how do the lab "single complexes" relate to atmospheric aerosols? Once these*



*three are answered you might consider a cloud parameterization but, as it currently stands, none of the three are described in detail. This needs to be corrected if this "cloud parameterization" is to remain in the paper.*

This has already been dealt with, see above. For this purpose the abstract, "introduction", and "summary and conclusion" sections have been re-written.

In them we now more clearly demonstrate the general applicability of our parameterization also to *Pseudomonas syringae* bacterial cells being atmospheric relevant and potential ice nucleation active.

"Other points" # 1:

*CRITICAL : Previous study results should be contained for comparison in one or more figures. I note the data of Wood in Figure 8 but most obviously this should be done in Figure 3 regardless of if the size might be somewhat different. The reader needs to be allowed to compare to what is already known and what is new here. Ideally, data would also be shown in Figure 7 (i.e., in nucleation rate space).*

We do not agree with referee here. In our opinion, including results from other studies in, e.g., Fig. 3 and Fig. 7 would destroy the line of thought in the manuscript and be misleading. Generally, existing data is now more thoroughly considered and discussed throughout the manuscript. Additionally, often datasets can not be easily compared with our data. This concerns datasets obtained with the drop freezing method suggested by Vali (1971), which usually are reported as cumulative ice nuclei concentrations, and for which often information on the cell concentrations in the examined droplets, which are needed for the conversion to ice fractions, are missing. This is included in the manuscript, now (see section 3.4).

*Introduction (30): "...soot belong to these major constituents of ice crystal residues (e.g., Pratt et al. (2009); Kamphus et al. (2010); Twohy and Poellot (2005)" With the exception of a portion of the last reference these papers actually DON'T support soot as a major IN (mineral dust, yes). Please find other references although I think actual data supporting soot as an IN is rare outside a few lab studies.*

The referee is correct concerning the minor or non importance of soot acting as IN. Since it is not an important statement for the paper it was deleted from the manuscript.

*Introduction (34): "Ice nucleating active (INA) bacteria, being ubiquitous in the atmosphere..." to my knowledge while INA bacteria have been FOUND in the atmosphere data do not indicate they are UBIQUIOUS. These are two VERY different terms. None of the references state they are ubiquitous, indeed the first reference (Moehler et al. 2008) cites a need for more studies in atmospheric abundance. This statement is not supported by the literature and needs to be toned down.*

We considered this when rewriting the introduction. The corresponding sentence now reads: "Morris et al. (2004) found that *P. syringae* was ubiquitously present in precipitation and freshwater and has suggested that INA bacteria are being disseminated as a part of the water cycle."

*Within 4.2 Poisson distribution of identical IN (215): Unless I'm missing something it seems likely that the abundance of "complexes" relates to the concentration of SNOMAX within the atomizer whereas this section discusses the relation to droplet volume. Further, as pointed out above, the reader is left unconvinced that complexes don't relate to the production method of SNOMAX which could be subject to change*



*from batch to batch. I think some statements here need to be made to make it clear the generation method and concentration of material is inherent in what is found about the distribution of IN. This should be explained in more detail. To be clear, as pointed out in line 233 the higher volume particles have more ice active material but the concentration chosen also determines this. Indeed it isn't until line 325 that the statement of* $10^4$ *cells per droplet is made.*

In our experiments, the average number of INA protein complexes per particle, at given dry particle size, is independent of the concentration of Snomax$^{TM}$ in the solution/suspension used for particle production. This is because the generated particles are dried after atomization and then size selected. Changes in the concentration of the Snomax$^{TM}$ solution/suspension affect the dry particle size distribution in terms of number of particles at a specific mobility diameter. But the mass of the dry particle, and consequently the average number of INA protein complexes (assuming no demixing) per particle at a given size does not change. The average number of protein complexes per Snomax$^{TM}$ particle, however, might differ between different Snomax$^{TM}$ batches due to slightly different production condition and therefore efficiency in producing INA protein complexes. But, again the number of INA protein complexes per particle, or in other words per Snomax$^{TM}$ mass, is accounted for in our approach.

This is now explained in the text in more detail. Especially, the volume/mass dependence of the average number of INA protein being present in the particles/droplets is now described in section 3.2.

*Summary (425): "We found that INA protein complexes controlling the ice nucleation behavior of Pseudomonas syringae bacteria belong to the most active IN considered up to now." More so than AgI? It would seem these complexes are often, if not always, somewhat less effective than some of the man-made cloud seeding agents. Specifically within LACIS or by all groups world-wide? Statement on editing: In general*

*a very well written paper but will need to be edited for English. Numerous dropped punctuation marks and small grammatical errors.*

We removed the statement from the manuscript in course of re-writing the "summary and conclusion" section.

We hope most of the errors are eliminated in the revised version of the manuscript.



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