Atmos. Chem. Phys. Discuss., 14, C11525–C11554, 2015
www.atmos-chem-phys-discuss.net/14/C11525/2015/

Atmospheric Chemistry and Physics Discussions

Interactive comment on "Ice nucleation by water-soluble macromolecules" *by* B. G. Pummer et al.

B. G. Pummer et al.

b.pummer@mpic.de

Received and published: 25 January 2015

We thank Referee #2 for his comments and suggestions concerning our manuscript. Below, we present our responses. We hope that the critical points were addressed sufficiently.

COMMENT: I completely agree with RC C8428 that this paper reads like a review article in places. I cannot comment much on whether the data are only supplementary to other published works, rather than warranting publication outright. It seems there are potentially some new findings in the paper; however, as presented results were not explained coherently enough to be able to judge.

General I enjoyed reading the introduction. I felt it gave a very nice introduction to the

area. However, it is a little bit long for a research article and reads a little bit like a thesis in places. It is almost as if a table would help to summarise some of the findings for different sources of ice nucleating macromolecules.

RESPONSE: Indeed, the introduction may look like a mini-review, however, the rest of the paper does not fulfill this format, since our main drive is to present new data and ideas. The introduction has the purpose of helping our readers, which come from very different fields and backgrounds, to understand our motivations, thoughts and conclusions. We shortened p.24276, L.23 - p.24280, L.25 and converted it into its own section in the discussion (now Sect. 4.2). A further shortening of the paragraph would lead to the loss of valuable information, which is essential for our arguments in the latter chapters. An overview of the central findings is shown in Table 1.

COMMENT: In general the whole of the Methods section 2.1 could be made much clearer. I found it hard to follow a lot of it. For a specific statement see, page 24282, lines 10-26 and next page lines 1-5. I struggled to understand what was being said here. What is the "proper dilution"? Also, please explain all the variables in Equation 1 and the meaning of them more clearly. nm for instance is not really defined. I would like to see an explanation of where the equation comes from.

RESPONSE: We rewrote several lines of the chapter. For a better overview, we only list the most relevant changes here, while we do not list minor exchanges of words:

p.24281, L.7-12: replaced with "High-purity water (18.2 MΩâĂćcm) was tapped from a water purification system (Thermoscientific™ Barnstead GenPure xCAD plus), autoclaved at 394 K for 20 min, and filtrated through a sterile 0.1 μm PES filter (Corning™). Then 10 ml of the high-purity water were added to the mycelium in the tube, which was then shaken with a vortex device (VWR™ lab dancer) three times for 30 seconds and filtrated through a 5 μm PES syringe filter (Acrodisc®), yielding a transparent solution."

p.24282, L.10-12: expanded "To determine the IN concentration per gram of mycelium,

the same setup and procedure as in Fröhlich-Nowoisky et al. (2014) were applied: Each sample was diluted with ultrapure water to an INM concentration where Eq. (1) gives finite results (the proper dilution was determined by trial and error)."

p.24282, L.22: inserted "nm is the number of INMs per gram of mycelium" and deleted "is" after "fice".

The formula is a variation of the Vali formula, which assumes that the freezing of droplets is a first order reaction, since every droplet is isolated and freezes with a certain statistical probability. The additional factors scale the probability with the INM content per droplet.

p.24283, L.1-5: replaced with " We note that Eq. (1) assumes that each droplet contains the same number of IN, i.e. the mean number of IN. However, at very small concentrations, the distribution of INMs in the droplets follows Poisson statistics, so that even for a mean number of one INM per droplet some droplets may contain two or more INMs and others no INMs at all. Without the use of Poisson statistics all of these would be counted as one in the analysis (Augustin et al., 2013)."

p.24283, L.10: inserted "This is done by determining values for θ and σ such that the measured values of fice are reproduced by the model. The corresponding equation describing the contact angle distribution and the Soccer Ball Model are given explicitly in Niedermeier et al. (2014)."

p.24283, L.15-18: replaced with "The concept of contact angles has, in the past, been applied for ice nucleating particles consisting of mineral dust, for which reasonable results were obtained (see e.g. Niedermeier et al., 2014). Here we apply it to describe the ice nucleation induced by water-soluble INMs, and we were able to derive contact angle distributions such that all measured data can be reproduced by the Soccer Ball Model. More specifically, a contact angle distribution determined for a sample reproduced all measurements done for that sample, even if different concentrations, different cooling times or completely different measurement approaches, as those described in

the following paragraphs, were used."

p.24283, L.19-22: expanded paragraph "INA was also measured with two additional experimental techniques. For both setups, 0.1 μm filtrates that were prepared as described at the top of this section were diluted and applied. These two additional methods were included to expand the data to lower temperatures, which was possible due to the smaller droplet sizes these methods examined (BINARY droplet volumes are about 1 μl) and to ensure that a possible interaction between the examined droplets and the substrates did not influence the results (LACIS examined freely floating droplets). Resulting values for nm are compared to the nm derived from the conventional freezing droplet array. Those systems are:"

Fröhlich-Nowoisky, J., Hill, T. C. J., Pummer, B. G., Franc, G. D., and Pöschl, U.: Ice nucleation activity in the widespread soil fungus Mortierella alpina, Biogeosci. Discuss., 11, 12697–12731, doi:10.5194/bgd-11-12697-2014, 2014.

COMMENT: In the next line (page 24283, line 6) the paper then talks about the soccer ball model; however, it is not clear how equation 1 relates to this treatment. This should be made clearer. The paper then talks about 2 extra methods using drop freezing array and the LACIS. There should be some justification as to why you use these different methods, to inform the reader why you are doing this.

RESPONSE: The respective equations describing the Soccer Ball Model are not reproduced in this text, as we feel that this is too detailed and not really needed. If you find this necessary, please let us know and we can add them. For the time being, the following was added explicitly to the text (p.24283, L.10): “[. . .] filtrate curves. This is done by determining values for ïĄś and ïĄş such that the measured values of fice are reproduced by the model. The corresponding equation describing the contact angle distribution and the Soccer Ball Model are given explicitly in Niedermeier et al. (2014).”

The extra methods were used to expand the data-set which were obtained with the 50 ïĄl aliquots. The freezing array examined smaller droplets (about 1 ïĄl), which

reduced the background (see Fig. 3). Larger droplets/aliquots increase the probability of contamination of the sample due to ice nucleating matter included in the water (ever ultrapure water still carries a few ice nucleating entities in it, so that 50 ïAl). LACIS was included as this is the only one of the three methods which examines free floating droplets, i.e. data for ice nucleation is obtained without interference with any substrate. This was done to ensure that the substrates (a glass slide for the freezing array and the wells for the method used in Mainz) did not influence the results. We added to the text (p.24283, L.19): “These two additional methods were included to expand the data to lower temperatures, which was possible due to the smaller droplet sizes these methods examined (BINARY droplet volumes are about 1 μl) and to ensure that a possible interaction between the examined droplets and the substrates did not influence the results (LACIS examined freely floating droplets).”

COMMENT: Section 3 is very hard to follow. I tried to work my way through this section, but in the end I gave up trying. As an example of something that isn't clear see page 24288, line 27 “Consequently, the ice nucleation temperatures are maximum a few Kelvin above the homogeneous freezing temperature (see Fig.4).” Figure 4, however, does not appear to show anything about maxima in ice nucleation temperatures. Does it not just show that PVA nucleates ice at just lower than 240 K?

RESPONSE: We rewrote several of the paragraphs, the most important of which are listed below:

p.24285, L.1-3: replaced with "In addition, aliquots of the birch pollen extracts digested with Trypsin and medium before and after incubation were forwarded through a Size Exclusion Chromatography (SEC) column, and the different eluted fractions were tested for their INA. Details about the setup and the measurements are presented in Sect. S2.2. This way, we checked if the enzymatic treatment changed the mass range of the birch pollen INMs."

p.24286, L.11-22: replaced with " Fig. 3 shows the comparison between the data from

BINARY, LACIS, and the droplet freezing array (Sect. 2.1). Each strain shows a relatively good overlap of the plateaus obtained with the different methods. Only when comparing the C-strain measurements, a difference in total nm can be seen, which, however, is less than one order of magnitude. The initial freezing temperatures are higher for the conventional droplet freezing array in Mainz in comparison with BINARY. This may indicate that the investigated INMs show a small time-dependence, which would lead to an increase in nm at lower temperature for the experiment with the larger cooling rate (i.e. BINARY), in agreement with the observations. From that it becomes evident that onset temperatures, which were often reported in the past, do not properly describe the ice nucleation process. They depend on the detection limit of the measurement method, as well as the INM content per droplet, and they are influenced by impurities or statistical outliers. Hence, the temperature at which 50% of all droplets froze (T50) was taken for interpretation."

p.24287, L.3-6: replaced with "After the elution from the SEC column, small amounts of INMs were spread across all fractions of the eluate. This might be caused by the adhesion of the organic matter in the extracts to the column packing, what undermines the separation principle. The tendency for adhesion of organic matter from pollen was already investigated by Pummer et al. (2013b). "

p.24287, L.17: shifted up chapter 3.3 and deleted headline ("INA of BINM peptides")

p.24287, L.19-23: replaced with "In Fig. 4, we plot the experimentally determined molecular masses of INMs against the observed ice nucleation temperature. For comparison, we show the theoretical parameterization of the ice embryo size by Zobrist et al. (2007), which is based on Classical Nucleation Theory. The sources of the plotted data are specified in Table 3."

p.24288, L.4-7: replaced with "We deduce that these free biological INMs which carry a suitable hydration shell mimic a theoretical ice embryo of the same size well enough to show the same INA. However, ice embryos of this size are almost impossible to

form spontaneously, what explains the low temperatures that are necessary for homogeneous ice nucleation. In contrast, the biological INMs have a given shape, what explains their high INA."

p.24288, L.10-29: cut short and replaced with "Both PVA and BINMs consist of a sequence of monomers covalently linked to each other. Longer chains fold into compact three-dimensional structures. Without any further forces, polymers coil randomly. Therefore, confined geometries do not exceed the size of a few monomers, where it is the limited flexibility of the monomer-to-monomer bond that enforces certain geometries. Hence, an increase in the total INM mass will not increase its INA. In contrast, intact proteins have a strongly determined folding, which is held together by intramolecular forces, and sometimes even forced on them by folding-supporting proteins. Therefore, a native protein's structure is stabilized in a certain geometry, as is the molecular surface. The unfolding of a biological macromolecule – a process called denaturation – changes many of its properties. This is also valid for the INA of INMs, and explains their deactivation by heat far below the temperatures where the covalent molecular bonds are broken. It is also responsible for the destruction of most INMs by the chaotropic guanidinium chloride. Summed up, randomly coiled INMs like PVA allocate only small, one-dimensional templates for ice nucleation (Fig. 1b) and therefore nucleate ice at rather low temperatures (Fig. 4)."

p.24289, L.16-21: replaced with "The 10 mg/ml sample showed only homogeneous ice nucleation. The 30 mg/ml sample showed an initial freezing temperature at about 250 K, a flat slope of nm(T) towards lower temperatures, and a T50 between 240 and 245 K in different experiments. The variance is rather high, since the aggregate formation seems to be very sensitive to the handling of the sample."

COMMENT: Section 4.1 does not really present the findings in a coherent way. It is a discussion rather than conclusion. I would suggest that, if the authors still want to present a research article, a separate / concise conclusions section is needed to present the new ideas.

RESPONSE: We renamed chapter 4 "Discussion" instead of "Discussion / Conclusions" and added another chapter 5 "Conclusions"

COMMENT: A lot of Section 4.2 is pure speculation. I was hoping for a focussed conclusions section that explained the findings, perhaps in bullet points

RESPONSE: With section 4.2, we indeed speculate what implications our findings might have on the atmosphere, and want to encourage further field and model studies that put effort in answering these questions. We do not consider it too speculative, since we backed up all our claims with studies, or connected them with basic considerations (e.g. the build-up of a cell). To express stronger that we do not intend to forestall future studies addressing this topic, we renamed the chapter "Potential atmospheric effects" instead of only "Atmospheric impacts"

Following the suggestion, we added another chapter "Conclusions" with the following content:

"Even free water-soluble macromolecules are able to nucleate ice, since they are in the same size range as the ice embryos necessary for ice formation. INMs can be diverse in chemical structure and origin, which may range from biopolymers in primary biological aerosols (proteins, saccharides, lipids, hybrid compounds), to secondary organic aerosol components (HULIS, etc.), to synthetic polymers (PVA). The allocation of functional groups, as well as the confinement that keeps them in place, is essential for the efficacy of the INMs. An increase of the template size that can be realized by aggregation of single molecules leads also to an enhancement of the INA. In this study we have shown that the water-soluble INMs from the fungal species A. implicatum and I. farinosa are proteins, and we have obtained additional evidence that the birch pollen INMs are polysaccharides without relevant protein content. Water-soluble INMs are released by a wide range of biological species. They may be associated not only with primary biological aerosols but also with other atmospheric aerosol particles such as soil dust or sea spray. The potential effects of such INMs should be considered and

pose an additional challenge in the quantification and assessment of the importance of biological ice nucleation in the atmosphere."

COMMENT: Methods, line 6. You say the mycelium was scratched off and 10 mL of high-purity water was added. What was the weight percent of the mycelium in the water? For example in experiments with mineral particles this is often quoted (e.g. in the experiments of Murray et al, 2011). Was the mycelium a powder or solid lumps?

RESPONSE: Depending on the harvest, it ranged from 0.6% to 8.6%. For normalization, we divided by the dry mass of the mycelium in Eq. 1, which eliminates the influence of the weight percent on nm. The mycelium showed the consistency of cotton (I. farinosa) or lumps (A. implicatum).

COMMENT: What was the reason for autoclaving and how do you this will affect your results?

RESPONSE: It is a standardized procedure for denaturation of biological cells and molecules in order to rid the water of possible contaminants.

COMMENT: When you pass through the 0.1 micron filter, what do you estimate to be the mass loading?

RESPONSE: We never investigated it, since we were more interested in the fraction that passes the filter.

COMMENT: Page 24283, line 16. When you say CNT works perfectly, what is your metric for this statement? It should be justified.

RESPONSE: Thank you for pointing out that our choice of wording was not precise enough, here. We have changed the corresponding lines (L.15-18) accordingly, and they now are as follows: “The concept of contact angles has, in the past, been applied for ice nucleating particles consisting of mineral dust, for which reasonable results were obtained (see e.g. Niedermeier et al., 2014). Here we apply it to describe the ice nucleation induced by INMs, and we were able to derive contact angle distributions such that

the measured data can be reproduced by the Soccer Ball Model. More specifically, a contact angle distribution determined for a sample reproduced all measurements done for that sample, even if different concentrations, different cooling times or completely different measurement approaches, as those described in the following paragraphs, were used.”

COMMENT: Page 24282, line 6 “no proteins” should be “not proteins”? There are a few typos throughout.

RESPONSE: We changed it as suggested.

COMMENT: Figure 2, font is too small. Legend is hard to interpret too, without delving into the text (i.e. it isn’t explained in the figure caption what the different lines mean). This makes it very difficult to follow what is being said in Sectoin 3.1, lines 1-10.

RESPONSE: We split up each diagram into 2 separate diagrams with only the 0.1 μm curve as common reference. We updated the figure caption: "Figure 2: nm(T)-curves for A. implicatum, I. farinosa, and M. alpina (subgroup D) INMs after different treatments. “G.Cl” stands for guanidinium chloride treatment, “B.A.” for boric acid treatment. A reduction in nm suggests that this method partly or fully destroyed the INMs. The absence of data points despite the listing in the figure legend indicates that nm lied below the detection limit. For M. alpina, the data are the mean curves of all investigated strains of the phylogenetic subgroup D, which is the most representative (Fröhlich-Nowoisky et al., 2014). The absence of a curve in a diagram means that no droplets were frozen at all. *) for A. implicatum and I. farinosa: these 0.1 μm measurements were executed with the filtrates of another harvest, as were the 5 μm and the B.A. measurements, what explains the higher values in comparison to the other results."

COMMENT: Could the authors comment on the following point, which affects the key findings of the paper. On page 24285, line 20 you say that the filtrate are particle free, because you don’t see particles in suspension. How do you know they are indeed

particle free, and it is just that you can't see the small particles in suspension?

RESPONSE: We investigated the residues left in the water in former studies with SEM (e.g. Pummer et al., 2012, 2013), where the absence of larger structures was demonstrated. Additionally, the Vivaspin filters only allow very small (molecular) components pass through them. It is a topic of debate where to draw the line between a molecule / particle in suspension and in solution for a macromolecule, since even a single dissolved molecule can be nanometers in size, and therefore be interpreted as a nanoparticle. In our former papers, we described the INMs as "suspended", since it is in better agreement with the conventional view of IN. The formal definitions of where to draw the line, however, strengthen the perspective of viewing it as a solution. The criteria are e.g. the absence of optical effects (e.g. scattering) in the range of visible light and the long-term phase stability (no sedimentation, no effect of centrifugation). From a molecular point of view, a molecule is in solution, if it is fully covered in a hydration shell. This is also valid for water-soluble proteins, while insoluble proteins flock together and sediment. Therefore, proteins can be regarded as dissolved despite their size (e.g. Osborne, 1910, Macedo, 2005). We also label our samples as "particle-free" to point out the difference to what is conventionally understood as particles: For mineral dusts, salts, soot, etc., a particle does not consist of a single formulaic unit, but of a large lattice respectively aggregate of a huge number of units (an elementary cell or an ion pair).

Macedo, E. A.: Solubility of amino acids, sugars, and proteins, Pure Appl. Chem., 77, 559-568, doi:10.1351/pac200577030559, 2005.

Osborne, T. B.: Die Pflanzenproteine, Ergebnisse der Physiologie, 10, 47-215, 1910.

Pummer B.G., Bauer H., Bernardi J., Bleicher S., Grothe H. (2012): Suspendable macromolecules are responsible for ice nucleation activity of birch and conifer pollen; Atmospheric Chemistry and Physics 12, p.2541-2550

Pummer B.G., Bauer H., Bernardi J., Chazallon B., Facq S., Lendl B., Whitmore K.,

Grothe H. (2013): Chemistry and morphology of dried-up pollen suspension residues; Journal of Raman Spectroscopy 44, p.1654-1658

We inserted a further paragraph into chapter S1.1 in the supplement, p.2, L.24:

"In the case of large molecules, it is disputable where to draw the line between solution and suspension. Per definition, a solution consists of a single phase, while a suspension consists of two phases with phase interfaces. If the particles sizes are close to the wavelength of visible light, a suspension shows light scattering, which makes it opaque. A solution, in contrast, shows neither light scattering, nor visible particles. Furthermore, a solution shows no phase separation over time, while sedimentation or agglutination lead to a progressive phase separation in time. Additionally, solutions cannot be separated by centrifugation. From a molecular point of view, a molecule in solution is fully covered with an energetically favorable hydration shell. Despite its size, a protein is a single molecule, while insoluble suspended particles consist of either several molecules or a crystal lattice of elementary cells."

COMMENT: Figure 3. I have the same issues with Figure 3 to Figure 2. Namely, the legend key and figure caption do not help me understand the figure. Is there really good agreement between the different methods? The 42C sample is coloured yellow for the MPIC method and red for the BINARY method and there is a large difference in the results. In fact I couldn't follow the meaning of the legend at all.

RESPONSE: We now show the full acronyms of the setups in the legend instead of using a one letter abbreviation. The data measured with the setup described in Fröhlich et al. (2014), which has no acronym, was labeled with an asterisk. The figure caption was rewritten: "Comparison of ice nucleation curves of 0.1 μm filtrates from a few M. alpina strains. The number and letter combination labels the strain. The devices used for generating the respective curves are shown in brackets. "*" stands for the setup described in Fröhlich-Nowoisky et al. (2014)."

APPENDIX: List of changes:

p.24275, L.4-16: we rewrote the abstract

"Cloud glaciation is critically important for the global radiation budget (albedo) and for initiation of precipitation. But the freezing of pure water droplets requires cooling to temperatures as low as 235 K. Freezing at higher temperatures requires the presence of an ice nucleator, which serves as a template for arranging water molecules in an ice-like manner. It is often assumed that these ice nucleators have to be insoluble particles. We point out that also free macromolecules which are dissolved in water can efficiently induce ice nucleation: The size of such ice nucleating macromolecules (INMs) is in the range of nanometers, which corresponds to the size of the ice embryo. As the latter is temperature-dependent, we see a correlation between the size of INMs and the ice nucleation temperature as predicted by Classical Nucleation Theory. Different types of INMs have been found in a wide range of biological species and comprise a variety of chemical structures including proteins, saccharides, and lipids. We combine new measurement results and literature data on INMs from fungi, bacteria, and pollen with theoretical calculations to foster a chemical perspective of ice nucleation and water-soluble INMs."

p.24275, L.19-21: replaced with "Consequently, supercooled droplets of ultrapure water stay liquid, until temperatures as low as 235 K are reached. The spontaneous self-assembling of water molecules in an ice-like arrangement, which is necessary for freezing to occur, is called homogeneous ice nucleation (see Fig.1a). "

p.24275, L.25: deleted "as"

p.24276, L.2: replaced "surface of the IN" with "IN surface"

p.24276, L.10-11: replaced with "In fact, soluble compounds consisting of ions, such as salts, or very small molecules, such as sugars and short-chained alcohols, cause a depression of the thermodynamic freezing point and the homogeneous ice nucleation temperature (Koop, 2004)."

p.24276, L.12: inserted "a large" before "enough surface"

p.24276, L.14: inserted "More information about INMs is given in Sect. S1.1"

p.24276, L.14: deleted "organic"

p.24276, L.16-17: replaced with "Further information about the ice nucleation process is compiled in the supplement (Sect. S1.2, S1.3, and S1.4)." combined 2 paragraphs

p.24276, L.18: replaced "among a variety of organisms" with "in various forms of life"

p.24276, L.20: shifted "in almost all cases" to the end of the sentence, inserted "regarded"

p.24276, L.21-22: we expanded the statement to "Overall, proteins, higher saccharides, and lipids, as well as hybrid compounds can play a role in INA, both as singular molecules as well as in aggregated form (Table 1, Sect. 4.2)."

p.24276, L.23: deleted "In the case of bacteria, it is a certain class of lipoglycoproteins."

p.24276, L.23 - p.24280, L.19: shifted; now chapter 4.2 ("Previous and recent findings on biological INMs")

p.24280, L.20-21: deleted "Fungi are abundant and diverse in the atmosphere (Fröhlich-Nowoisky et al., 2009, 2012). Therefore, their potential for atmospheric ice nucleation has to be regarded."

p.24280, L.22-25: replaced with " In this study, we characterize the water-soluble INMs found in the fungal species Acremonium implicatum and Isaria farinosa and we compare the results with other recent studies of water-soluble INMs from the fungus Mortierella alpina (Fröhlich-Nowoisky et al., 2014), from birch pollen (Pummer et al., 2012), and from bacteria (Niedermeyer et al., 2014). We also discuss relevant key findings of related earlier studies on the INA of biological materials (e.g. Govindarajan and Lindow, 1988a). Combining these data with calculations derived from Classical Nucleation Theory (Zobrist et al., 2007), we draw conclusions about the nature, sources, and

potential atmospheric effects of biological INMs."

p.24281, L.6: inserted "either" before "a scalpel"

p.24281, L.7-12: replaced with "High-purity water (18.2 MΩâĂćcm) was tapped from a water purification system (Thermoscientific™ Barnstead GenPure xCAD plus), autoclaved at 394 K for 20 min, and filtrated through a sterile 0.1 μm PES filter (Corning™). Then 10 ml of the high-purity water were added to the mycelium in the tube, which was then shaken with a vortex device (VWR™ lab dancer) three times for 30 seconds and filtrated through a 5 μm PES syringe filter (Acrodisc®), yielding a transparent solution."

p.24281, L.15: deleted "later"

p.24282, L.2: replaced "so" with "therefore"

p.24282, L.10-12: expanded "To determine the IN concentration per gram of mycelium, the same setup and procedure as in Fröhlich-Nowoisky et al. (2014) were applied: Each sample was diluted with ultrapure water to an INM concentration where Eq. (1) gives finite results (the proper dilution was determined by trial and error)."

p.24281, L.16: inserted "changes in" before "their INA"

p.24281, L.17: replaced "The change of INA" with "This"

p.24282, L.13: deleted 2x "then"

p.24282, L.19: replaced "so" with "therefore"

p.24282, L.20: deleted "see"

p.24282, L.22: inserted "nm is the number of INMs per gram of mycelium" and deleted "is" after "fice".

p.24283, L.1-5: replaced with " We note that Eq. (1) assumes that each droplet contains the same number of IN, i.e. the mean number of IN. However, at very small

concentrations, the distribution of INMs in the droplets follows Poisson statistics, so that even for a mean number of one INM per droplet some droplets may contain two or more INMs and others no INMs at all. Without the use of Poisson statistics all of these would be counted as one in the analysis (Augustin et al., 2013)."

p.24283, L.10: inserted "This is done by determining values for θ and σ such that the measured values of fice are reproduced by the model. The corresponding equation describing the contact angle distribution and the Socccer Ball Model are given explicitly in Niedermeier et al. (2014)."

p.24283, L.13-15: replaced with "[...] and added literature data for INMs from birch pollen (Augustin et al., 2013) and bacteria (Niedermeier et al., 2014)."

p.24283, L.15-18: replaced with "The concept of contact angles has, in the past, been applied for ice nucleating particles consisting of mineral dust, for which reasonable results were obtained (see e.g. Niedermeier et al., 2014). Here we apply it to describe the ice nucleation induced by water-soluble INMs, and we were able to derive contact angle distributions such that all measured data can be reproduced by the Soccer Ball Model. More specifically, a contact angle distribution determined for a sample reproduced all measurements done for that sample, even if different concentrations, different cooling times or completely different measurement approaches, as those described in the following paragraphs, were used."

p.24283, L.19-22: expanded paragraph "INA was also measured with two additional experimental techniques. For both setups, 0.1 μm filtrates that were prepared as described at the top of this section were diluted and applied. These two additional methods were included to expand the data to lower temperatures, which was possible due to the smaller droplet sizes these methods examined (BINARY droplet volumes are about 1 μl) and to ensure that a possible interaction between the examined droplets and the substrates did not influence the results (LACIS examined freely floating droplets). Resulting values for nm are compared to the nm derived from the conventional freezing

droplet array. Those systems are:"

p.24283, L.23: replaced "A freezing droplet array called" with "A droplet freezing array termed"

p.24283, L.25-26: replaced with "A detailed description of the technique, the preparation of droplets, and the data acquisition and evaluation is given in Budke and Koop (2014)."

p.24284, L.6-8: replaced with "To test the hypotheses that birch pollen INMs are polysaccharides and not proteins (Pummer et al., 2012), further procedures for characterization of the birch pollen INMs were carried out."

p.24284, L.15-20: replaced with "First, boric acid was added to an aliquot of fungal extract to a concentration of 0.75 M. The aliquot was left overnight at room temperature, as boric acid is known to esterify with sugars. This treatment should alter the INA of the birch pollen INMs, in case that saccharides play a role. However, since the esterification process does not necessarily affect all functional groups, the INA might be only partially eliminated. Since the INA assay preparation has a certain statistical uncertainty, minor changes in the INA are difficult to interpret."

p.24284, L.24-26: replaced with "To check if birch pollen INMs are indeed non-proteinaceous, three separate 100 μl aliquots were mixed with 94 μl of (i) water, (ii) medium without enzyme, (iii) medium with trypsin, and incubated for 18 h at 310 K."

p.24285, L.1-3: replaced with "In addition, aliquots of the birch pollen extracts digested with trypsin and medium before and after incubation were forwarded through a Size Exclusion Chromatography (SEC) column, and the different eluted fractions were tested for their INA. Details about the setup and the measurements are presented in Sect. S2.2. This way, we checked if the enzymatic treatment changed the mass range of the birch pollen INMs."

p.24285, L.4: renamed chapter "Ice nucleation experiments with bacterial INM pep-

tides"

p.24285, L.6: replaced "Ps. syringae BINM" with "bacterial INM (BINM) of Pseudomonas syringae"

p.245285, L.16: deleted "/discussion"; chapter headline is "Results"

p.245285, L.24: deleted "see"

p.24286, L.5: inserted "approximately" before "100 to 300"

p.24286, L.11-22: replaced with "Fig. 3 shows the comparison between the data from BINARY, LACIS, and the droplet freezing array (Sect. 2.1). Each strain shows a relatively good overlap of the plateaus obtained with the different methods. Only when comparing the C-strain measurements, a difference in total nm can be seen, which, however, is less than one order of magnitude. The initial freezing temperatures are higher for the conventional droplet freezing array in Mainz in comparison with BINARY. This may indicate that the investigated INMs show a small time-dependence, which would lead to an increase in nm at lower temperature for the experiment with the larger cooling rate (i.e. BINARY), in agreement with the observations. From that it becomes evident that onset temperatures, which were often reported in the past, do not properly describe the ice nucleation process. They depend on the detection limit of the measurement method, as well as the INM content per droplet, and they are influenced by impurities or statistical outliers. Hence, the temperature at which 50% of all droplets froze (T50) was taken for interpretation."

p.24286, L.24: inserted "for the trypsin test" after "medium"; deleted "however"

p.24286, L.25: deleted "at all"

p.24286, L.26: inserted "from the medium" after "formic acid"

p.24286, L.27: replaced "medium" with "respective measurement"

p.24287, L.3-6: replaced with "After the elution from the SEC column, small amounts

of INMs were spread across all fractions of the eluate. This might be caused by the adhesion of the organic matter in the extracts to the column packing, what undermines the separation principle. The tendency for adhesion of organic matter from pollen was already investigated by Pummer et al. (2013b). "

p.24287, L.10: replaced "would make" with "makes"

p.24287, L.13-14: replaced "critical cluster" with "ice embryo"

p.24287, L.17: shifted up chapter 3.3 and deleted headline ("INA of BINM peptides")

p.24287, L.18: expanded headline for chapter 3.2 ("Comparison with theoretical calculations of the ice embryo size")

p.24287, L.19-23: replaced with "In Fig. 4, we plot the experimentally determined molecular masses of INMs against the observed ice nucleation temperature. For comparison, we show the theoretical parameterization of the ice embryo size by Zobrist et al. (2007), which is based on Classical Nucleation Theory. The sources of the plotted data are specified in Table 3."

p.24287, L.25: replaced "critical cluster" with "ice embryo"

p.24288, L.4-7: replaced with "We deduce that these free biological INMs which carry a suitable hydration shell mimic a theoretical ice embryo of the same size well enough to show the same INA. However, ice embryos of this size are almost impossible to form spontaneously, what explains the low temperatures that are necessary for homogeneous ice nucleation. In contrast, the biological INMs have a given shape, what explains their high INA."

p.24288, L.10-29: cut short and replaced with "Both PVA and BINMs consist of a sequence of monomers covalently linked to each other. Longer chains fold into compact three-dimensional structures. Without any further forces, polymers coil randomly. Therefore, confined geometries do not exceed the size of a few monomers, where it is the limited flexibility of the monomer-to-monomer bond that enforces certain geome-

tries. Hence, an increase in the total INM mass will not increase its INA. In contrast, intact proteins have a strongly determined folding, which is held together by intramolecular forces, and sometimes even forced on them by folding-supporting proteins. Therefore, a native protein's structure is stabilized in a certain geometry, as is the molecular surface. The unfolding of a biological macromolecule – a process called denaturation – changes many of its properties. This is also valid for the INA of INMs, and explains their deactivation by heat far below the temperatures where the covalent molecular bonds are broken. It is also responsible for the destruction of most INMs by the chaotropic guanidinium chloride. Summed up, randomly coiled INMs like PVA allocate only small, one-dimensional templates for ice nucleation (Fig. 1b) and therefore nucleate ice at rather low temperatures (Fig. 4)."

p.24288, L.29: inserted "long-range" before "confined"

p.24289, L.1-2: deleted 2x "see"

p.24289, L.5-7: deleted "Of course, the surface of these 2-D-templates has to be properly functionalized in order to arrange the water molecules, or else they show no INA at all."

p.24289, L.8: deleted headline ("INA of BINM peptides") and shifted chapter up to p.24287, L.17

p.24289, L.10: deleted "In view of Fig.4"

p.24289, L.16-21: replaced with "The 10 mg/ml sample showed only homogeneous ice nucleation. The 30 mg/ml sample showed an initial freezing temperature at about 250 K, a flat slope of nm(T) towards lower temperatures, and a T50 between 240 and 245 K in different experiments. The variance is rather high, since the aggregate formation seems to be very sensitive to the handling of the sample."

p.24290, L.1: deleted "and conclusions"

p.24290, L.2: renamed chapter "Solubility of INMs"

p.24290, L.6-7: replaced with "Furthermore, any ice nucleating template requires a certain size to be able to support a critical ice embryo that is large enough to grow into a macroscopic crystal."

p.24290, L.13: deleted "in the supplement"

p.24290, L.14: inserted "protein" before "molecules", replaced "lower sugars" with "low molecular weight saccharides"

p.24290, L.16: replaced "be" with "act"; added "active" before "molecular surface"

p.24290, L.19: replaced "solution" with "solution's"

p.24290, L.26 - p.24291, L.2: replaced with "We therefore emphasize that a more molecular view on IN allows a better understanding of the process of heterogeneous ice nucleation. We see a link between this molecular view and the macroscopic view that is necessary for developing atmospheric models. For example, the contact angle is a macroscopic interpretation of the molecular interaction between phases."

p.24291, L.6: replaced "be" with "cover"

p.24291, L.8-9: replaced "[...] or by a large hydration shell around these INMs that has to be added to the total IN mass." with "[...] or by the ability of forming a larger hydration shell that has to be taken into account."

p.24291, L.18: inserted parts from introduction (p.24276, L.23 - p.24280, L.19) as chapter 4.2., entitled "Previous findings on biological INMs". Cut it short and inserted paragraph about HULIS, SOA and organic acids. Full text:

"The already mentioned BINMs that have been found so far are a certain class of bacterial lipoglycoproteins that are fully sequenced and characterized (e.g. Abe et al., 1989). In some cases, biological INMs of one type or species show more than one freezing temperature in an ice nucleation spectrum. This variation in INA can be explained by the presence of different functional groups, foldings or aggregation states

(e.g. Govindarajan and Lindow, 1988a, Augustin et al., 2013, Dreischmeier et al., 2014, this study). The presence of INMs seems to have certain advantages, which might be the motivation for certain species to produce them (Sect. S1.5). The bacterial gene is highly conserved and codes for a 120 kDa β-helical membrane protein with many repeated octapeptides (Green and Warren, 1985, Abe et al., 1989, Kajava and Lindow, 1993, Schmid et al., 1997, Graether and Jia, 2001, Garnham et al., 2011). The INA induced by this protein also involves glycosides and lipids that stabilize it in the outer membrane of the bacterial cell and assure its conformation for an optimum functioning (Kozloff et al., 1984, Govindarajan and Lindow, 1988a, Turner et al., 1991, Kawahara, 2002). With the side chains, the total mass of a single BINM is about 150–180 kDa (Table 1). It is assumed that the initiation point for ice formation is the amino acid sequence TXT in the repeated octapeptide, where T designates threonine and X any other amino acid. The OH groups of the two threonine moieties match the position of oxygen atoms in the ice lattice. Since a BINM contains several of these sequences at positions and distances that correspond to the ice lattice structure it can stabilize an ice embryo and so decrease the activation barrier for ice nucleation (Graether and Jia, 2001). As sequence modification studies on a structurally related antifreeze protein have shown, the loss of the TXT has a devastating effect on the interaction with water molecules, while other modifications have a much weaker impact (Graether et al., 2000). The expression of BINMs is an exclusive property of certain bacterial species. It has been reported for a wide range of strains in the P. syringae species complex (Lindow et al., 1982, Berge et al. 2014), P. fluorescens and borealis (Fall and Schnell, 1985, Obata et al., 1987, Foreman et al., 2013), Erwinia uredovora (Obata et al., 1990a), Pantoea agglomerans, formerly called E. herbicola (Phelps et al., 1986,), Pantoea ananatis (Coutinho and Venter, 2009), Xanthomonas campestris (Kim et al., 1987), a Pseudoxanthomonas sp. (Joly et al., 2013), and more. The efficacy of their INA depends on both the strain and the cultural growth conditions, e.g. the available nutrients and the growth temperature (Rogers et al., 1987, Nemecek-Marshall et al., 1993, Fall and Fall, 1998). In most cases, these BINMs are aggregated and anchored in the outer cell membrane, where

the strength of the INA depends on the aggregation state and the chemistry of the membrane (Govindarajan and Lindow, 1988a+b, Kozloff et al., 1991). However, free BINMs still show appreciable INA, although less than in the native state (Schmid et al., 1997). Since these complexes match the ice crystal lattice perfectly, these bacteria are the most active IN known at present. These anchored aggregates of BINMs on the otherwise ice nucleation inactive cell surface are a demonstrative example of active sites on a larger IN, which is the micro-sized bacterial cell. In some cases, bacteria release their active sites carried on much smaller membrane vesicles. These are spherical pieces of the outer cellular membrane that are excised from the cell, a natural and common phenomenon in bacteria in general (Deatherage and Cookson, 2012). The expression of such vesicles with BINMs has been reported for Pantoea agglomerans / E. herbicola (Phelps et al., 1986), E. uredovora (Kawahara et al., 1993), and P. fluorescens (Obata et al., 1993). P. syringae and viridiflava express such BINM-carrying vesicles only under certain growth conditions (Obata et al., 1990b, Pooley and Brown, 1990). For P. putida, the INA found in culture supernatants was associated with a 164 kDa lipoglycoprotein and had activity both as an IN and as an antifreeze protein. In this case, removal of the approximately 92 kDa of carbohydrates eliminated the INA, however, not the antifreeze properties (Xu et al., 1998). INMs were also found in the kingdom of fungi (Kieft, 1988, Kieft and Ahmadjian, 1989, Kieft and Ruscetti, 1990, Pouleur et al., 1992, Hasegawa et al., 1994, Tsumuki and Konno, 1994, Tsumuki et al., 1995, Richard et al., 1996, Humphreys et al., 2001, Morris et al., 2013, Haga et al., 2013, Fröhlich-Nowoisky et al., 2014). Similarly to the bacteria, only a limited fraction of investigated strains showed INA, while the majority was inactive (Pouleur et al., 1992, Tsumuki et al., 1995, Iannone et al., 2011, Pummer et al., 2013a, Huffman et al., 2013, Fröhlich-Nowoisky et al., 2014). Fungal INMs can be divided into two subgroups, both of which differ from the BINMs. The INMs of rust fungi show properties of polysaccharide compounds (Morris et al., 2013). The already characterized INMs from Rhizoplaca chrysoleuca (Kieft and Ruscetti, 1990), F. avenaceum (Pouleur et al., 1992, Hasegawa et al., 1994, Tsumuki and Konno, 1994), and M. alpina (Fröhlich-Nowoisky et al., 2014)

are evidently proteins, but show barely any other similarities with the BINMs. They are more tolerant to stresses, have a different amino acid sequence, seem to have less to no lipid and carbohydrate functionalizing, and are easily released from the cells. Only recently, a 49 kDa protein from F. acuminatum was suggested as being the INM (Lagzian et al., 2014). Proteins and lipoproteins with INA were also found in extracellular fluids of insects like Tipula trivittata larvae (Duman et al., 1985, Neven et al., 1989, Duman et al., 1991, Warren and Wolber, 1991), Vespula maculata queens (Duman et al., 1984), and Dendroides canadensis larvae (Olsen and Duman, 1997). The only non-proteinaceous insect INs found up to date are phosphate spherules and fat cells in the larvae of Eurosta solidaginis (Mugnano et al. 1996). INs have also been detected in other animal taxa, e.g. amphibians (Wolanczyk et al., 1990) and mollusks (Aunaas, 1982, Hayes and Loomis, 1985, Madison et al., 1991, Lundheim, 1997), as well as in spider silk (Murase et al., 2001). The fluid reservoirs of some succulent plants, namely Lobelia telekii and Opuntia species, contain polysaccharide INMs (Krog et al., 1979, Goldstein and Nobel, 1991, Goldstein and Nobel, 1994). Other reported non-proteinaceous plant INs are from the wood of Prunus species (Gross et al., 1988), or the lignin in a waste water sample (Gao et al., 1999). Among plant INs, only those of Secale cereale were identified as proteins (Brush et al., 1994). The pollen of some plant species showed appreciable INA in different lab studies, among which that of silver birch (Betula pendula or alba) was the most active one (Diehl et al., 2001, Diehl et al., 2002, von Blohn et al., 2005, Pummer et al., 2012, Augustin et al., 2013). The birch pollen contain easily extractable, very robust INMs, which are non-proteinaceous and most likely some type of polysaccharide (Pummer et al., 2012). The extracts were characterized via vibrational spectroscopy, which indicated that they contained sugar-like compounds, proteins, and other biological molecules, but no sporopollenin, which is the fabric of the outer pollen wall (Pummer et al., 2013b). Other organic aerosols in the focus of ice nucleation research are Humic-Like Substances (HULIS) and Secondary Organic Aerosols (SOAs). They show certain similarities to the presented INMs, since they consist of a large variety of organic macromolecules that have undergone com-

plex biochemical processing. Analogously, several exponents showed little to no INA in experiments, or even oppressed INA in mixed particles by blocking the active sites (e.g. Möhler et al., 2008, Prenni et al., 2009), while others showed appreciable INA. Certain HULIS standards (Wang and Knopf, 2011) and some SOAs (Wang et al., 2012, Schill et al., 2014) induced ice nucleation in the deposition and the immersion mode. The O/C-ratio of the latter did not affect the INA, although it influenced several other properties, such as the kinetics of the water uptake, in agreement with recent model simulations (Berkemeier et al., 2014). Among glassy aerosols composed of saccharidic components, some chemical species showed significant INA that might even compete with mineral dust INA in mid-latitude clouds (Wilson et al., 2012). Even a simple compound like citric acid shows INA when it is in the state of a glassy aerosol (Murray et al., 2010). The inorganic salt ammonium sulfate possesses INA in the crystalline state in both the immersion and deposition mode, despite it being a highly soluble compound (Zuberi et al., 2001, Abbatt et al., 2006)."

p.24291, L.19: replace headline "4.2. Atmospheric impacts" with "4.3. Potential atmospheric effects"

p.24291, L.23: replaced "that" with "which"

p.24291, L.25: inserted "however" before "the detection"

p.24291, L.25: inserted "despite their size" at the end of the sentence

p.24292, L.6: replace "frequently found" with "present"

p.24292, L.7: replace "Amato et al., 2007" with "Vaitilingom et al., 2012"

p.24292, L.10: deleted "furthermore"

p.24293, L.5: inserted discussion about modeling and a chapter for the conclusions:

"Several former studies aimed at quantifying biological INs either by analyzing precipitation samples (Christner et al., 2008a+b), or by atmospheric modeling based on

emission and deposition data (Hoose et al., 2010). In both cases, however, only whole cells were regarded. Christner et al. (2008a+b) filtered the particles of interest out of the samples, and so lost the molecular fraction, to which the INMs we described belong. Hoose et al. (2010) did not include fragmentation or phase separation processes that can release molecular compounds from the carrier particles in the atmosphere. This might have led to an underestimation of biological INs in the results.

5. Conclusions

Even free water-soluble macromolecules are able to nucleate ice, since they are in the same size range as the ice embryos necessary for ice formation. INMs can be diverse in chemical structure and origin, which may range from biopolymers in primary biological aerosols (proteins, saccharides, lipids, hybrid compounds), to secondary organic aerosol components (HULIS, etc.), to synthetic polymers (PVA). The allocation of functional groups, as well as the confinement that keeps them in place, is essential for the efficacy of the INMs. An increase of the template size that can be realized by aggregation of single molecules leads also to an enhancement of the INA. In this study we have shown that the water-soluble INMs from the fungal species A. implicatum and I. farinosa are proteins, and we have obtained additional evidence that the birch pollen INMs are polysaccharides without relevant protein content. Water-soluble INMs are released by a wide range of biological species. They may be associated not only with primary biological aerosols but also with other atmospheric aerosol particles such as soil dust or sea spray. The potential effects of such INMs should be considered and pose an additional challenge in the quantification and assessment of the importance of biological ice nucleation in the atmosphere."

p.24293, L.18: inserted "Abbatt, J. P. D., Benz, S., Cziczo, D. J., Kanji, Z., Lohmann, U., and Möhler, O.: Solid ammonium sulfate aerosols as ice nuclei: a pathway for cirrus cloud formation, Science, 22, 313, 1770-1773, doi:10.1126/science.1129726, 2006."

p.24293, L.19: deleted Amato et al. (2007)

p.24294, L.9: inserted "Berkemeier, T., Shiraiwa, M., Pöschl, U. and Koop, T.: Competition between water uptake and ice nucleation by glassy organic aerosol particles, Atmos. Chem. Phys., 14, 12513–12531, doi:10.5194/acp-14-12513-2014, 2014."

p.24295, L.23: deleted Fröhlich et al. (2009)

p.24295, L.26: deleted Fröhlich et al. (2012)

p.24298, L.22: inserted "Koop, T.: Homogeneous Ice Nucleation in Water and Aqueous Solutions, Zeitschrift für Phys. Chemie, 218, 1231-1258, doi:10.1524/zpch.218.11.1231.50812, 2004."

p.24299, L.8: inserted "Möhler, O., Benz, S., Saathoff, H., Schnaiter, M., Wagner, R., Schneider, J., Walter, S., Ebert, V., and Wagner, S.: The effect of organic coating on the heterogeneous ice nucleation efficiency of mineral dust aerosols, Environ. Res. Lett., 3, 025007, doi:10.1088/1748-9326/3/2/025007, 2008."

p.24299, L.22: inserted "Murray, B. J., Wilson, T. W., Dobbie, S., Cui, Z., Al-Jumur, S. M. R. K., Möhler, O., Schnaiter, M., Wagner, R., Benz, S., Niemand, M., Saathoff, H., Ebert, V., Wagner, S., and Kärcher B.: Heterogeneous nucleation of ice particles on glassy aerosols under cirrus conditions, Nat. Geosci., 3, 233-237, doi:10.1038/ngeo817, 2010."

p.24301, L.1: inserted "Prenni, A. J., Petters, M. D., Faulhaber, A., Carrico, C. M., Ziemann, P. J., Kreidenweis, S. M., and DeMott, P. J.: Heterogeneous ice nucleation measurements of secondary organic aerosol generated from ozonolysis of alkenes, Geophys. Res. Lett., 36, L06808, doi:10.1029/2008GL036957, 2009."

p.24301, L.20: inserted "Schill, G. P., De Haan, D. O., and Tolbert, M. A.: Heterogeneous ice nucleation on simulated secondary organic aerosol, Environ. Sci. Technol., 48, 1675-1682, doi:10.1021/es4046428, 2014."

p.24302, L.5: inserted "Vaitilingom, M., Attard, E., Gaiani, N., Sancelme, M., Deguillaume, L., Flossmann, A. I., Amato, P., and Delort, A.-M.: Long-term features of cloud

microbiology at the puy de Dôme (France), Atmos. Environ., 56, 88-100, 2012."

p.24302, L.13: inserted "Wang, B., and Knopf, D. A.: Heterogeneous ice nucleation on particles composed of humic-like substances impacted by O3, J. Geophys. Res., 116, D03205, doi:10.1029/2010JD014964, 2011." and "Wang, B., Lambe, A. T., Massoli, P., Onasch, T. B., Davidovits, P., Worsnop, D. R., and Knopf, D. A.: The deposition ice nucleation and immersion freezing potential of amorphous secondary organic aerosol: Pathways for ice and mixed-phase cloud formation, J. Geophys. Res., 117, D16209, doi:10.1029/2012JD018063, 2012."

p.24302, L.13: inserted "Wilson, T. W., Murray, B. J., Wagner, R., Möhler, O., Saathoff, H., Schnaiter, M., Skrotzki, J., Price, H. C., Malkin, Dobbie, S., and Al-Jumur, S. M. R. K.: Atmos. Chem. Phys., 12, 8611-8632, doi: 10.5194/acp-12-8611-2012, 2012."

p.24302, L.28: inserted "Zuberi, B., Bertram, A. K., Koop, T., Molina, L. T. and Molina, M. J.: Heterogeneous Freezing of Aqueous Particles Induced by Crystallized (NH4)2SO4 , Ice, and Letovicite, J. Phys. Chem. A, 105, 6458–6464, doi:10.1021/jp010094e, 2001."

Table 1: replaced "yes" and "no" with plus and minus; replaced "algae" with "different algae"; replaced "interrogation mark" with "question mark"; deleted "some" before "uncertainty" deleted first "the" in figure caption; replaced "temperature about which" with "temperatures above which"; replaced "Introduction" with "Sect. 4.2"

Figure 2: diagrams were replaced; new figure caption: " Figure 2: nm(T)-curves for A. implicatum, I. farinosa, and M. alpina (subgroup D) INMs after different treatments. “G.Cl” stands for guanidinium chloride treatment, “B.A.” for boric acid treatment. A reduction in nm suggests that this method partly or fully destroyed the INMs. The absence of data points despite the listing in the figure legend indicates that nm lied below the detection limit. For M. alpina, the data are the mean curves of all investigated strains of the phylogenetic subgroup D, which is the most representative (Fröhlich et al., 2014). The absence of a curve in a diagram means that no droplets were frozen

at all. *) for A. implicatum and I. farinosa: these 0.1 μm measurements were executed with the filtrates of another harvest, as were the 5 μm and the B.A. measurements, what explains the higher values in comparison to the other results."

Figure 3: format and labeling improved; new figure caption: "Comparison of ice nucleation curves of 0.1 μm filtrates from a few M. alpina strains. The number and letter combination labels the strain. The devices used for generating the respective curves are shown in brackets. "*" stands for the setup described in Fröhlich et al. (2014)"

List of changes in the supplement:

p.1, L.18: replaced "so" with "thereby"

p.2, L.5: inserted "it"

p.2, L.12: inserted "given"

p.2, L.25: inserted an additional paragraph to discuss insoluble particles vs. solution:

"In the case of large molecules, it is disputable where to draw the line between solution and suspension. Per definition, a solution consists of a single phase, while a suspension consists of two phases with phase interfaces. If the particles sizes are close to the wavelength of visible light, a suspension shows light scattering, which makes it opaque. A solution, in contrast, shows neither light scattering, nor visible particles. Furthermore, a solution shows no phase separation over time, while sedimentation or agglutination lead to a progressive phase separation in time. Additionally, solutions cannot be separated by centrifugation. From a molecular point of view, a molecule in solution is fully covered with an energetically favorable hydration shell. Despite its size, a protein is a single molecule, while insoluble suspended particles consist of either several molecules or a crystal lattice of elementary cells."

p.3, L.17: replaced "critical cluster" with "ice embryo"

p.3, L.19: inserted "arranged" after "45000"; replaced "critical cluster" with "ice embryo"

p.4, L.6: replaced "therefore decreasing" with "and therefore decreases"

p.4, L.7: shifted "(INs)" before "and"

p.5, L.4: replaced "at" with "on"

p.5, L.20 - p.6, L.5: replaced all "aW" with "aw"

p.5, L.22: replaced "the" with "insoluble"

p.6, L.29: replaced "have" with "were"

p.7, L.6: replaced "are" with "is"

p.7, L.7: deleted "however"

p.8, L.10: replaced "Zolles, 2013" with "Zolles et al., 2015"

p.8, L.18-19: replaced ",which [...] its" with ". That [...] the"

p.8, L.26: inserted "(AFP)" after "protein"

p.9, L.8: replaced "in" with "by"

p.9, L.27: corrected format: "-1" is superscript

p.13, L.26-27: replaced Zolles et al. (2013) quote with:

"Zolles, T., Burkart, J., Häusler, T., Pummer, B., Hitzenberger, R., and Grothe, H.: Identification of ice nucleation active sites on feldspar dust particles, J. Phys. Chem. A, accepted, doi:10.1021/jp509839x, 2015."

Interactive comment on Atmos. Chem. Phys. Discuss., 14, 24273, 2014.