Response to Referee 1

The referee's original comments are in italics, and our response follows.

This work extends an immersion ice nucleation model to account for mixtures of differ- ent aerosol species present within the droplets. Freezing experiments are performed with Snowmax and illite and analyzed using the so-called critical area framework. The authors place emphasis on the influence of external vs. internal variability defining ice nucleation temperatures. They also investigate whether the freezing properties of an illite-Snomax mixture can be described using the contact angle distributions obtained from the individual species.

The correct representation of the immersion freezing of cloud droplets is critical for atmospheric modeling. New frameworks that account for the full range of variability observed in nature are certainly within the scope of ACP. Unfortunately the proposed model seems inconsistent with its own premises and may not lead to a better under- standing of the freezing processes. The model assumptions are not clearly stated and the main equations are presented without much justification. The experimental proce- dure is confusing and lightly described. The paper is also difficult to read and follow. Everywhere there is superfluous information that does not seem relevant and that dis- tracts from the main points of the work. My feeling is that a full overhaul of the paper and even of the underlying model and experiments may be required. At this point the work is not suitable for publication in ACP.

We thank the reviewer for their extensive comments that have helped us improve the clarity and conciseness of our manuscript. We think that much of the reviewer's concerns regarding the model and its expanded form can be addressed via clarifying important aspects in the manuscript under revision and by emphasizing and clarifying the formulation of our original model presented by Beydoun et al. (2016). As repeating what was presented in that original paper in this manuscript is not appropriate, we have strived to direct the referee to the relevant sections of the original paper to address their specific questions, and also clarified this manuscript to make it easier to understand how the model is constructed and used in this study. We note that many of the referee's questions were addressed in the original 2016 paper, while the focus of this new manuscript is not on the model's development and justification, but rather on its application to understanding the ice nucleation properties of Snomax, and Snomax-illite mixtures.

General comments

In general the paper is full with confusing and convoluted statements in places where simple sentences would suffice. The authors should consider reducing its length by re- moving repeated statements and unnecessary explanations. Also please be more precise. Many ambiguous concepts are thrown out without explanation and just add con- fusion to the text (e.g., activity, externally-mixed diversity, freezing capabilities, ice nucleation ability, ice nucleation critical temperatures, characteristic temperatures, droplet systems). Although I have tried to point out *some of them, I'd encourage the authors to put a lot more emphasis on simplicity and easiness of reading.*

Thank you for pointing out these issues that reduce the clarity of the manuscript. The wording in many places has been changed to avoid the overuse of terms that describe the same thing. This will be highlighted in the responses to detailed comments below. Heterogeneous ice nucleating (HIN) activity is consistently used to describe the ice nucleating strength of a particle type. External/internal variability are defined in a good amount of detail in Sect. 1.2 and are a measure of how the distribution of HIN activity distributes itself among a particle population. In the revised manuscript these terms have been omitted and replaced with other terms that are now clearly defined and used throughout the manuscript: ice nucleation ability, ice nucleation critical temperatures, and characteristic temperatures. We use the term "freezing temperatures" to describe the temperatures at which droplets freeze.

Please shorten the abstract and emphasize the main points. The first paragraph should be removed.

This suggestion is addressed in the response to the first specific comment below.

The model itself seems inconsistent. Particle sometimes refers to droplets with embed- ded aerosol, sometimes to the aerosol itself, sometimes to the droplets. The procedure used to obtain the distributions and each of the freezing curves must be much more clearly explained. It seems inconsistent that n_draws is treated as a fitting parameter. Normally as n_draws increases g→g however the authors manage to do just the op- posite: higher n_draws produce more variability and a further diverging g* from g. This casts doubt on the consistency of the model. It is also not clear that what is described in the paper is exactly what is done.*

The model assumes that for a given particle concentration, each droplet contains the same particle surface area. This is discussed in detail in the original description of the model published in ACP by Beydoun et al. (2016). Above the critical area, each particle surface is assigned a g distribution equal to $\bar{g}$. Below the critical area each particle surface is assigned a g distribution g^* which is generated from random sampling of contact angles from $\bar{g}$. This process is repeated for each particle surface so that below the critical area particle surfaces exhibit different g^* distributions. In the revised manuscript we have added more clarity and explanation of the original premises of the model but we emphasize the importance of looking at our prior publication by Beydoun et al. (2016) published in ACP for a more extensive explanation and analysis of the model framework. The reviewer states that we have increased variability with higher n_{draws}, but this is certainly not the case. For example, when sampling from the first distribution of HIN of Snomax the text states the following (Page/line 15/24-25):

"The consequent n_{draws} for each frozen fraction curve between the two Snomax critical areas are: 80 for $3\text{x}10^{-2}$ wt%, 65 for $1\text{x}10^{-2}$ wt%, 40 for $5\text{x}10^{-3}$ wt%, 25 for $1\text{x}10^{-3}$ wt%, and 6 for $5\text{x}10^{-4}$ wt%."

More specific details on this concern are further addressed in the responses to other specific comments below, along with the revisions we made to address these issues.

Everywhere: please use standard scientific notation.

We have switched to standard scientific notation. That certainly makes the manuscript more readable.

There is a complete omission on the effect of variations in the aerosol surface area per droplet in the droplet population. This is contradictory to the main premise of the model where some droplets contain enough material to be above the critical area and others don't. This is a major inconsistency and the authors should explain how it is resolved.

The issue of surface area variability between particles at the same concentration is an important issue that was addressed in the original description of our g framework in Beydoun et al. (2016). We found that surface area variability could not explain the changes in the droplet freezing temperature spectra as concentration was decreased. For a given concentration our model assumes all droplets contain the same surface area. The surface area varies with mass concentration. Thus there is no contradiction to the main premise of the model. We have clarified the explanation of the model in section 2.2 to better communicate the working premises of the model, as follows on Page/Line 9/1-20 and 10/1-11:

"In Beydoun et al. (2016) we started from classical nucleation theory to formulate a framework of heterogeneous ice nucleation which states that any given particle surface can be assigned a distribution of HIN activity, g. g is a continuous normal distribution of contact angles, θ (used as a proxy for the HIN activity at the particle surface-water interface), and determines the probability that a particle induces freezing in a droplet, P_f, at a temperature, T, via:

$$P_f(T) = 1 - \exp\left(-tA\int_0^{\pi} g(\theta)J(\theta,T)d\theta\right) \qquad (1)$$

where t is the time the droplet spent at the temperature T, A is the surface area of the particle, and $J(\theta, T)$ is the heterogeneous ice nucleation rate per unit surface area per unit time as defined in Zobrist et al. (2007). The freezing probability can also be written for a droplet undergoing a constant cooling rate $\dot{T}$ as:

$$P_f = 1 - \exp\left(-\frac{A}{\dot{T}}\int_{T_i}^{T_f}\int_{0}^{\pi} g(\theta)J(\theta,T)d\theta dT\right) \qquad (2)$$

For a large ensemble of droplets containing identical particle surfaces, the freezing probability is equal to the fraction of droplets frozen, F.

We hypothesized that an individual particle type possesses a critical surface area, above which one normal distribution of HIN activity $\bar{g}$ can accurately describe the freezing probability of a droplet containing any particle of that type. Therefore, the freezing probability of a droplet containing a particle with a surface area larger than this critical area can be evaluated using Eqns. (1) or (2) with $\bar{g}$ in place of g and the fraction of droplets frozen, F, of a large ensemble of these droplets N is equal to the freezing probability of each droplet, P_f. On the other hand, when a particle possesses a surface area below that type's critical surface area a random sampling of contact angles to generate a discrete distribution of HIN activity g^* is required for each particle surface in the particle population. In this case the frozen fraction of a large ensemble of droplets, F, is the arithmetic mean of the individual droplet freezing probabilities and can be evaluated using:

$$F = \frac{1}{N}\sum_{i=1}^{N} P_{f,i}\left(g^{*}{}_{i}(n_{draws})\right) \qquad (3)$$

where $P_{f,i}$ is the freezing probability of droplet i and can be evaluated using Eqns. (1) and (2) with g^* used in place of g and n_{draws} is the number of contact angle draws made from $\bar{g}$. The effect of decreasing an INP's total surface area below its critical surface area threshold on the distribution of HIN activity contained in each droplet is illustrated in Fig. 1 by the extensive broadening of the droplet freezing temperature curve when below the critical area. Above the critical area the diversity of HIN activity is essentially eliminated across the particle population; the large available surface area guarantees that the full distribution of HIN activity is contained in each droplet and described by□The creation of the g^* distribution by taking n_{draws} from $\bar{g}$ accounts for this increased diversity in HIN activity when below the critical area threshold."

The authors never seem to consider that classical nucleation theory (CNT) has very significant flaws, and the underlying physics may be wrong. This is particularly true for biological material that does not conform at all with the assumption of a gas-like nucleation process embedded in CNT.

The model uses the mathematical formulation of CNT and adds free parameters to predict heterogeneous ice nucleation behavior. This approach has been undertaken by many groups in the ice nucleation community (e.g. Alpert and Knopf, 2016; Broadley et al., 2012; Niedermeier et al., 2011; Welti et al., 2012; Wright and Petters, 2013) and has proven to demonstrate a good degree of success in modelling the HIN activity of particle surfaces. In all these formulations the traditional CNT framework is modified by incorporating some distribution of contact angles instead of a single contact angle, or a distribution of particle surfaces (the approach of Alpert and Knopf, 2016). In doing so, we are no longer testing freezing behavior against CNT as enough free parameters exist to act as correction factors to the assumed bulk properties embedded in the free energy terms. That is why, despite our model's successful modelling of the HIN activity of Snomax, we never claim that this success gives support or validation to the ability of CNT to predict the freezing behavior of biological material.

To avoid confusion, we have reworded the text introducing the model in Sec. 2.2 (Page/Line 9/2-3):

"In Beydoun et al. (2016) we started from classical nucleation theory to formulate a framework of heterogeneous ice nucleation which states that any given particle surface can be assigned a distribution of HIN activity, *g*."

It is not clear whether illite and Snomax are mixed within the droplets but still remain externally mixed. Did the authors prepare Snowmax/illite particles that were then suspended? Otherwise little can be said on the intermediate range of freezing temperatures.

The mixed suspensions were prepared by mixing the Snomax and illite suspensions, and the droplets were then generated from that mixture. The external mixture exhibited in the frozen fraction curve corresponding to this mixture suggests that some of the droplets do not contain Snomax ice nucleating particles and thus freeze according to the HIN activity of illite. We have added text explaining how the mixed particle suspensions were prepared in Sect. 2.1 on Page/line 8/20-24:

"Suspensions from which the droplets are generated are prepared by mixing a known mass of the sample (Snomax or illite) with a known volume of ultrapure Milli-Q water to form a suspension with a specific weight percentage of the sample in water. The droplets containing a mixture of Snomax and illite were generated from a suspension formed by first creating a 1:1 mixture from the pure illite and pure Snomax suspensions."

Specific comments:

Page 1, Line 6-15. This discussion just distracts from the main points of the paper. It is also irrelevant since the authors do not study the budget and distribution of INP in the atmosphere nor their effect on cloud microphysics.

It is not unheard of in a study on INPs to mention their importance in cloud microphysics and mixed phase clouds. Motivating the work should not be seen as a distraction from the main points of the paper. If the reviewer means to say that such motivating details shouldn't be in the abstract, then we believe that is a matter of personal preference and style. Our understanding is that a generous introductory/motivating paragraph in the abstract is permissible and often desirable. Furthermore, it is ACP editorial policy to encourage longer extended abstracts for their manuscripts, and to briefly introduce the topic and its importance in the abstract.

Page 1, Line 16. The abstract should start around here, clearly enunciating the main points of the paper.

Please see our reply to the previous comment.

Page 2, Line 3. This seems to imply that Snowmax is not active at low temperature, which I find hard to believe. Please clarify

We provide very strong evidence that the freezing happening at low temperatures is due to the presence of illite when analyzing a mixture of Snomax and illite. This is due to the excellent agreement between the part of the frozen fraction curve corresponding to freezing due to Snomax and the frozen fraction curve due to pure illite. Further we (along with many groups before us) have identified a temperature cut off below which no additional HIN activity is induced. Recall that as ice nucleation is a singular event that any ice active sites that can freeze at a warmer temperature will have already done so before the colder temperature is reached. Thus Snomax can certainly cause ice nucleation at colder temperatures, but it will have already induced freezing before this temperature is reached. Please see Wex et al. (2015) Fig. 11 – below -12 C the ice active site density levels off as all the Snomax INP's have already induced freezing.

Page 2, Line 5. This may be inaccurate. It seems that no Snowmax-Illite mixtures were prepared, and they remain externally mixed within each droplet. Please clarify.

Please see our response above on mixture preparations – each droplet was generated from a mixture of Snomax and illite particles. For the droplets generated from a mixture, we can be certain that all droplets contain illite INPs but not all droplets contain Snomax INPs – that is the topic of investigation here. This is deduced from the frozen fraction curves whereby one portion of the curve aligned with a frozen fraction curve corresponding to pure Snomax while the other portion aligned with a frozen fraction curve corresponding to pure illite. The droplets that froze according to the HIN activity of Snomax did contain illite – this is demonstrated in an experiment where the same illite concentration is used to generate droplets containing pure illite and the droplets freeze in a well-defined temperature range (see frozen fraction corresponding to 0.05 wt% illite in Fig. 5).

Page 2, Lines 20-24. This is a very convoluted statement. Please correct it and make it more readable. I'll suggest something like: "Very few studies have focused on this temperature range".

The statement on Page/Line 2/20-24 has been changed and now reads:

"This is a temperature range where investigated mineral samples of atmospherically relevant particle sizes (or other atmospherically relevant non-biological particles) have not exhibited detectable ice nucleating activity (Atkinson et al., 2013; Cantrell and Heymsfield, 2005; DeMott et al., 2015; Murray et al., 2012)."

Page 3, Line 17. The word "new" seems out of place here.

Page 3, Line 17 in the ACPD manuscript doesn't contain the word "new".

Page 3, Line 21-23. This is vague. The statement is complete without the g framework reference.

The *g* reference has been removed from the statement. It now reads (Page/line 3/24 and 4/1-2):

"These mixed particle systems also provide a valuable opportunity to evaluate the ability of HIN models to predict the freezing properties of mixtures using the properties obtained experimentally from the pure components."

Page 4, Lines 3-13. These lines do not seem to meaningfully contribute to the dis- cussion and make the paragraph overly long. Defining internal variability based on a "couple of degrees" Kelvin is also ambiguous. I'd suggest removing the lines.

We agree with the reviewer that those can mostly be removed. The paragraph now reads (Page/line 4/4-18):

"In Beydoun et al. (2016) the concepts of internal variability and external variability in HIN activity were defined and shown to have a dramatic impact on the temperature range a droplet population freezes in. If the same distribution of HIN activity is contained within each particle in an aerosol population (the internal variability), then the range of temperatures over which these particles cause freezing in the droplets they are contained in would be significantly different than if that same distribution was distributed externally (the external variability). Figure 1a illustrates the concepts of internal versus external variability. In the top right-hand panel of Fig. 1a each droplet contains a particle surface with a color gradient representing the distribution of HIN activity. Note that each particle contains the same distribution of HIN – demonstrating full internal variability – and therefore the range of temperatures over which droplets freeze is relatively narrow, as depicted in the bottom panel. As the surface area of the particle surface in each droplet decreases, the HIN distribution becomes externally variable, meaning that each droplet now contains a different distribution of HIN activity, which is shown with the different colors the particle surfaces are illustrated with in the top left hand panel of Fig. 1a. The reduced surface area per droplet leads to a reduction in the freezing temperatures while the increased external variability leads to a broader temperature range over which droplets freeze relative to the larger surface area case where the variability in HIN activity between each droplet was eliminated."

Page 4, Line 17. The range of temperatures is "narrow" only if the distribution is also narrow. It is not necessarily a measure of internal variability.

In the critical area framework, when particle surfaces are above the critical area threshold the variability in freezing temperatures is attributable to internal variability. This hypothesis was fully explored by Beydoun et al. (2016).

Page 4, Lines 19-20. This sentence is out of place.

The sentence has been removed.

Pages 4-5. Line 25. This is a confusing statement. Please split this sentence.

We have clarified the writing and the sentence now reads (Page/line 4/16-19):

"The reduced surface area per droplet leads to a reduction in the freezing temperatures while the increased external variability leads to a broader temperature range over which droplets freeze. This is in contrast to the larger surface area case where the variability in HIN activity between each droplet was eliminated."

Figure 1, caption. Most of this is already explained in the section. Please reduce this wall of text.

The figure caption should explain all schematics used within the figure so it is clear to the reader without having to consult the main text. As this figure is comprised of two parts and each part comprises multiple scenarios, it is not possible for us to shorten the figure caption while still achieving this goal. We have tried to make it a bit more concise by omitting unnecessary explanations of the scenarios the figure depicts. The caption now reads (Page 5):

a) Top, Right: Droplets containing particle surfaces larger than the component's critical surface area exhibiting internal variability (color gradient) in HIN activity. **Left:** Droplets contain particle surfaces smaller than the component's critical surface area and exhibit external variability in HIN depicted by the different colors. **Bottom:** Decreasing surface area leads to higher external variability in HIN activity and thus a wider range over which droplets freeze. **b) Top, Right:** Droplets contain a mixture of mineral dust (brown) and biological (green) particle surfaces, both of which are larger than their component's critical surface area. **Left**: Two hypothetical scenarios of reduced biological particle surface areas are depicted as the concentration of biological INP is reduced. In (2) the biological material distributes itself equally among the droplets and thus remains internally mixed while in (3) the biological material does not further partition itself further and becomes externally mixed. Scenario (4) represents the absence of any biological material and the resultant temperature freezing spectrum is identical to the one for dust lying above its critical surface area shown in the bottom of Fig. 1a. **Bottom:** The predicted impact of the INP concentration and mixing state of the dust-bio mixture on the resultant freezing spectrum of droplets containing the particle mixture.

Page 5. Line 3. This paragraph although somehow relevant is also distracting and a detour from the main points of the work.

We prefer that the reader is exposed to some motivation for the importance of internal/external variability after the concepts are explained and therefore have retained this paragraph.

Page 6, line 10. Does this mean that each droplet has a different distribution of freezing efficiencies?

We prefer the use of the term distribution of "HIN activity" over "freezing efficiency". What this means is that one distribution of HIN activity does not suffice to model the freezing behavior of such a system. The sentence now reads:

"If an aerosol population is chemically diverse and this diversity is externally distributed, then the freezing capabilities of the entire particle population would need to be defined by multiple distributions of HIN activity."

Page 6, lines 8-20. Again this is a very confusing paragraph and it is not clear what it adds to the discussion. Most of this was said in the previous page.

We respectfully disagree with the referee; this paragraph discusses chemical mixing state for the first time in the manuscript. We have therefore retained this section as it adds important new discussion.

Page 7, first paragraph. This is very repetitive. The whole paragraph can be reduced to a single question. I'd say: Is there an intermediate range of freezing temperatures when two dissimilar aerosol populations are present within each droplet?

Reducing the paragraph to a single question such as the one the reviewer suggests would eliminate the explanation embedded on how the freezing temperatures of the droplets containing the mixture relate to internal/external variability of the INP's. This explanation is also that of the right hand panel of Fig. 1.

Page 8, Line 11. Does this imply a freezing fraction uncertainty of 0.02?

If the uncertainty is defined by the resolution of the produced frozen fraction, then yes. This detail is now mentioned in the revised text on Page/line 8/8-9:

"A solution of ultrapure milli-Q water and the particle material being investigated was prepared, from which 40-60 (thus a 0.02 resolution in the retrieved frozen fraction)."

Page 9, Equation 1. The upper limit in the integral should be π. This expression indicates that the "g" distributions pertains to the droplet not to the particle. If the authors assume that each droplet has one particle, please explicitly state it. Also the integral merely represents the average nucleation rate within the droplet.

Yes the upper limit should be π, that typo has been fixed, thank you for pointing this out. The expression pertains to the freezing probability a droplet has if it includes a particle with a g distribution of HIN activity. The text now reads (Page 9/5-8):

"g is a continuous normal distribution of contact angles, θ (used as a proxy for the HIN activity at the particle surface-water interface), and determines the probability that a particle induces freezing in a droplet, P_f, at a temperature, T, via:

$$P_f(T) = 1 - \exp\left(-tA\int_0^{\pi} g(\theta)J(\theta,T)d\theta\right) \qquad (1)\text{''}$$

Regarding what Eqn. (1) represents, the reviewer is referred to the derivation of this expression in Beydoun et al. (2016), specifically Sect. 3.0.

Page 9 Line 7. This is only true for identical droplets. The authors also seem to neglect *variations in the surface area per droplet.*

That is correct, the model neglects variation in surface area per droplet. This is an effect we considered in our original paper on the g framework (Beyoun et al, 2016). The text has been revised to indicate that the frozen fraction is only equal to the freezing probability if the particle surfaces within the droplets are identical to each other (Page 9/13-14):

"For a large ensemble of droplets containing identical particle surfaces, the freezing probability is equal to the fraction of droplets frozen, F."

Regarding neglecting variations in particle surface area between droplets, this is explained in Beydoun et al. (2016). This is a relevant quote from Sect. 3.3 of that paper:

"For the application of this model to cold plate data where droplets are prepared from a suspension of the species being investigated, the particle population in each droplet is treated as one aggregate surface (and thus one large particle) and a mean surface area value is assumed for the particle material in all the droplets in the array."

Page 9 line 8-12. Please be precise in the terminology. So far the freezing probability refers to droplets, not particles. The one-droplet one-particle assumption is also implied here.

The freezing probability should refer to droplets while the HIN activity should refer to particle surfaces. The revised text now reads (Page/line 9/15-20 and 10/1-12):

"We hypothesized that an individual particle type possesses a critical surface area, above which one normal distribution of HIN activity $\bar{g}$ can accurately describe the freezing probability of a droplet containing any particle of that type. Therefore, the freezing probability of a droplet containing a particle with a surface area larger than this critical area can be evaluated using Eqns. (1) or (2) with $\bar{g}$ in place of g and the fraction of droplets frozen, F, of a large ensemble of these droplets N is equal to the freezing probability of each droplet, P_f. On the other hand, when a particle possesses a surface area below that type's critical surface area a random sampling of contact angles to generate a discrete distribution of HIN activity g^* is required to produce the freezing behavior of a droplet containing this particle. In this case the frozen fraction of a large ensemble of droplets, F, is the arithmetic mean of the individual droplet freezing probabilities and can be evaluated using:

$$F = \frac{1}{N}\sum_{i=1}^{N} P_{f,i}\left(g^{*}{}_{i}(n_{draws})\right) \qquad (3)$$

where $P_{f,i}$ is the freezing probability of droplet i and can be evaluated using Eqns. (1) and (2) with g^* used in place of g and n_{draws} is the number of contact angle draws made from $\bar{g}$. The effect of decreasing an ice nucleant's total surface area below its critical surface area threshold on the distribution of HIN activity contained in each droplet is illustrated in Fig. 1 by the extensive broadening of the droplet freezing temperature curve when below the critical area. Above the critical area the diversity of HIN activity is essentially eliminated across the particle population; the large available surface area guarantees that the full distribution of HIN activity is contained in each droplet and described by $\bar{g}$. The creation of the $g*$ distribution by taking n_{draws} from $\bar{g}$ accounts for this increased diversity in HIN activity when below the critical area threshold."

Page 9 lines 12-15. Please note that for large n_draws g should become g_average. Again the authors mix droplet and particle and it is not clear what the g distribution represents.*

Please see the previous comment regarding mixing the terms particle and droplet. HIN activity always refers to the particle surface while freezing probability refers to the droplet that a particle surface is immersed in. As for the reviewer's comment on the convergence of $g*$ to $\bar{g}$ for large n_{draws}, we refer to Beydoun et al. (2016) where this is clearly stated in Sec. 3.3:

"It should also be noted that there is an n_{draws} value for each system above which the sampled distribution mimics $\bar{g}$. For example, when n_{draws} is 25 for illite, the retrieved distribution will produce a freezing curve equivalent to using $\bar{g}$."

Page 10, Line 1. What is N?

In the revised text N is defined on Page/line 9/19. It refers to the number of particle-droplet pairings.

Page 10, line 6. Please specify what is meant by "saturated".

"Saturated" has been replaced with "eliminated" in the revised text.

Page 10, Eq. 4. Please justify this expression. There seems to be a contradiction since the critical surface area is defined per-droplet while here it is used as defined per-species. Please clarify.

The critical surface area was always defined per particle type or species, not per particle-droplet pair. We hope that the clarifications made in Sect. 2.2 now lead to a clearer description of the model premises and assumptions.

Page 11, Eq. 5. This expression only takes into account variation in contact angle, while it *assumes that every droplet contains the same surface area of each species. The model does not seem self-consistent (see main comments above).*

As mentioned in our response to the previous comments, variation in surface area between particle surfaces at the same material concentrations is neglected. Thus in Eqn. (5) each particle type has one value for a surface area.

Page 11, line 11. Change ''chapter" for Section.

The typo has been fixed.

Page 12, line 14. Here and in many other places. Please use standard scientific notation.

We have switched to using scientific notation throughout.

Page 12, line 14. How is the particle surface area distributed in the droplet population?

Please see our responses to previous comments regarding surface area variability between particles at the same material concentration.

Page 13, line 8. Again, the particle surface area inside the droplets is likely non- uniformly distributed and it is not clear what the authors are referring to.

The assumption of one value of particle surface area per material concentration should be clear at this point in the revised manuscript.

Page 13, line 15. Please clarify what the surface area correction is.

The surface area correction was meant to refer to using the same g distribution but with the estimated surface area value for this particular material concentration. The text has been changed from referring to a surface area "correction" to merely saying after accounting for the change in particle surface area (Page/line 13/16-18):

"The g_2 distribution that fits this low concentration freezing curve is a different Gaussian function than the g_1 distribution that fits the first two high concentration freezing curves (after accounting for the change in particle surface area)."

Page 14, lines 4-8. These values seem problematic. In CNT the nucleation rate is a monotonically decreasing function of the contact angle. So the fact that $\mu 1 > \mu 2$ even though the first population freezes at a higher temperature is contradicting the underlying physics. Secondly, the second distribution is essentially monodisperse, and it can't explain the enhanced variability.

The values are not problematic due to the fact that freezing by distribution 1 is dictated by the rising tail of the $\bar{g}_1$ distribution. The reviewer is urged to read Sect. 3.2 of Beydoun et al. (2016) where this is discussed in detail and the concept of a nucleating area is introduced. In addition, the second distribution is not monodisperse in the strictest sense of the word (a Dirac-delta function) but does have a finite sigma. This is mentioned on Page 19/16-18:

"However, it should be noted that the existence of some broadness in the temperature over which droplets freeze throughout the regime falling below the second critical area does necessitate that $\bar{g}_2$ still has a finite σ, albeit a small one."

Page 15, Line 10-15. This seems contradictory. Shouldn't the random sampling always lead to a different result? In other words what is the variation in F from the randomness introduced by the model?

Random sampling does lead to a different result for each particle surface, but when a frozen fraction is calculated as the arithmetic mean of each freezing probability in a large ensemble the variability is eliminated. We hope this is clearly outlined in Sect. 2.2 of the revised manuscript. A more extensive explanation is also found in Sect. 3.3 of Beydoun et al. (2016).

Page 15, Line 15. It is not clear what the authors mean by "optimization of the dynamic range of ndraws". Please clarify.

We do not say "optimization of the dynamic range of n_{draws}". A dynamic range isn't optimized. The original text says: "This optimization is carried out manually as the dynamic range of n_{draws} is small enough such that a more comprehensive numerical optimization (e.g. a Monte Carlo simulation) was not found to be necessary." Dynamic range here is referring to the range of values of n_{draws} that lead to different frozen fraction outcomes. Again, much of this is explained in detail in Beydoun et al. (2016). Nevertheless, we have reworded this sentence in the revised manuscript to remove any ambiguity (Page/line 15/15-17):

"The optimization of n_{draws} is carried out manually as the range of n_{draws} that results in changes to the modelled frozen fraction curves is small enough such that a more comprehensive numerical optimization (e.g. a Monte Carlo simulation) was not found to be necessary."

Page 15 Line 20. For the smallest concentration: separate draws should always lead to a different outcome. So how reproducible is this?

Please refer to the comment above on reproducibility.

Page 16, Line 5. This is equivalent to saying that g^* *is changing with composition and contradicts the premises of the model. For* $\mu 1 = 0.62$ *choosing* $\theta>2.20$ *does not represent the distribution only its rightmost tail. In fact such a value is not even depicted in Figure 3. Also this value seems way too large for such efficient ice nucleating particles.*

We actually choose $\theta > 0.22$, not > 2.20 as was mistakenly stated in the text. Please excuse a very problematic typo, which has been corrected now. The referee is absolutely right that a value of 2.2 for limiting drawing of contact angles represents none of the distribution.

Page 17, Lines 8-12. These statements also signal something troublesome about the model. Drawing 8000 samples from a normal distribution would for all practical purposes reproduce the original distribution. In fact at ndraws≈20 an acceptable representation of a normal distribution is already obtained, even more so for such a narrow distribution. In other words given enough draws a consistent model would always produce $g2^*\approx g2average$*. So the question is why are the curves so dependent on ndraws? Why is ndraws treated as a fitting parameter?*

Yes, given enough draws a consistent model would always reproduce $\bar{g}$. The number of draws however is dependent on the width of the distribution. The reason $\bar{g}_2$ requires more draws than $\bar{g}_1$ is due to the fact that its narrower. As draws happen across the entire range of θ, the narrower the distribution the more draws are necessary to capture the distribution. For $\bar{g}_1, \sigma_1 = 0.05$, therefore the number of draws to reproduce $\bar{g}_1 \approx$ 3.14/(0.05) = 62.4. On the other hand, for $\bar{g}_2 \approx$ 3.14/0.0001=3140. This is why the number of draws from $\bar{g}_2$ is much larger.

n_{draws} is treated as a fitting parameter since it can create external variability between the modelled particle surfaces, particularly when drawing a small number of times. It's very important to note that the drawing process happens N times (number of particle surface-droplet pairs being modelled) so that a repeatable frozen fraction is retrieved. This frozen fraction is the average of all retrieved freezing probabilities as dictated by Eqn. (5).

Page 18-20. This explanation is very repetitive. Can these two pages be confined to a couple of sentences stating that there is overlap between the two distributions, so that the observed differences are dominated by σ *instead of* μ*?*

That is not the only message we are trying to convene in this part of the text. The section concludes with relating the wider $\bar{g}_1$ distribution to the finding of Polen at al. (2016) that the most active ice nucleants in Snomax are subject to degradation and a reduction in their activity over time. Nevertheless, the text has been made more concise and now reads (Page/line 19/19-24 and 20/1-11):

"The second notable feature in the contrast between the two Snomax distributions of HIN activity shown in Fig. 3 is that the mode of the less active $\bar{g}_2$ distribution ($\mu_2 = 0.52$) is actually at a smaller contact angle (i.e. more ice active) than that of the $\bar{g}_1$ distribution ($\mu_1 = 0.60$). This is not contradictory to the resultant freezing curves where the INP's represented by $\bar{g}_1$ cause freezing at several degrees warmer than those of $\bar{g}_2$. The freezing temperatures are actually determined by the ascending tail of the first distribution for the droplets containing high Snomax concentrations (dashed portion of the $\bar{g}_1$ curve shown in Fig. 3). The tail of the broader $\bar{g}_1$ distribution spreads into a lower (more active) contact angle range than the much narrower $\bar{g}_2$ distribution does. Further, the broader $\bar{g}_1$ distribution of HIN activity can be interpreted as being consistent with the finding in Polen et al. (2016) that the more active Type I protein aggregates (which are described by the first distribution, $\bar{g}_1$) are less stable over storage time and refreezes than the Type III protein aggregates (which are described by the second distribution, $\bar{g}_2$). A broader distribution implies that freezing is determined by a wider (more variable) range of HIN activity and thus a wider diversity of the INP's properties. The freezing induced by the more active $\bar{g}_1$ distribution is understood to be caused by rare and very active large proteins aggregates that would arguably be more susceptible to deterioration, weakening, and decay over time. This would produce a wider diversity in HIN activity, which would result in the broader $\bar{g}_1$ normal distribution of contact angles (Fig. 3) that we derived from analysis of the droplet freezing spectra in Fig. 2."

Page 20, lines 15-20. It is not clear how these mixtures are prepared. How is a mixture 1:1 but also 0.1% in illite and 0.00001 % in Snowmax? Are they mixed in the solid phase then diluted? What steps are taken to ensure that each droplet contains a mixture of the two components?

The preparation of the mixture solutions is now outlined in Sect. 2.1. No steps are taken to ensure that each droplet contains both types of INPs. The goal of the study is to explore the extent to which Snomax can partition its INPs so that upon dilution a contribution to freezing remains. It is seen that is not the case by the sharp cut off in freezing temperature between freezing caused by Snomax and freezing caused by illite.

Page 23, Lines 1-15. This paragraph does not seem relevant and it is quite confusing.

The paragraph was meant to discuss an academic thought experiment, in which the amount of illite surface needed to match the freezing capabilities of Snomax is calculated. To avoid distraction from the main points of the paper, it has been removed.

Page 24, Lines 1-5. The authors seem to be describing an externally mixed population of droplets containing Snowmax. For such a case the analysis presented here would not apply since the premise is that all droplets contain both species.

Droplets are generated from a mixture of Snomax and illite particles suspended in water. It is the intent of this study to explore what happens at low Snomax concentrations: does Snomax partition itself continuously and sustain its contribution to freezing? Or does Snomax limit its partioning and the droplets become externally mixed? The questions are asked clearly in Sect. 1.2 and explored in the experiments conducted and analyzed here.

Page 25, Lines 4-5. Please clarify what "doping illite. . . is limited by its ability to partition itself" means. Also only illite is investigated not every dust particle.

"Doping" here refers to Snomax INPs enhancing the HIN activity of the illite surface. Since Snomax is unable to partition its INPs at very low concentrations, the mixture becomes externally mixed and Snomax is unable to spread itself onto all illite surfaces. "Doping" has been replaced with "enhancing" in the revised manuscript.

That is correct, only illite is investigated here that is why the text explicitly mentions that the conclusions are drawn for this proxy mixture only: illite + Snomax.

The revised text now reads (Page 24/18-24 and 25/1-4):

"From the perspective of modelling multi-component heterogeneous ice nucleating particles, it remains a subject of further research to determine how many $\bar{g}$ distributions are necessary to encompass the freezing behavior of mixed particle systems at low temperatures where dust is known to be quite ice active. However, the work presented here supports the notion that two distributions of HIN activity are necessary to distinguish between the dust and biological regimes when illite and Snomax are used as proxies for these two types of INP. Regarding the motivating hypothesis that small biological particles can enhance the freezing capabilities of mineral dust surfaces, it can be concluded that the effectiveness of Snomax enhancing illite or any mineral dust surface is limited by its ability to partition itself and thus allow its HIN activity to be manifested externally. Hypothetical scenario 3, that was introduced in section 1.2 and depicted in Fig. 1b, in which the limited amount of biological material does not distribute itself among all droplets, therefore closely resembles the bio-dust mixture proxy examined here."

Cited References

Alpert, P. A. and Knopf, D. A.: Analysis of isothermal and cooling-rate-dependent immersion freezing by a unifying stochastic ice nucleation model, Atmos. Chem. Phys., 16(4), 2083–2107, doi:10.5194/acp-16-2083-2016, 2016.

Atkinson, J. D., Murray, B. J., Woodhouse, M. T., Whale, T. F., Baustian, K. J., Carslaw, K. S., Dobbie, S., O'Sullivan, D. and Malkin, T. L.: The importance of feldspar for ice nucleation by mineral dust in mixed-phase clouds, Nature, 498(7454), 355–358, doi:10.1038/nature12278, 2013.

Beydoun, H., Polen, M. and Sullivan, R. C.: Effect of particle surface area on ice active site densities retrieved from droplet freezing spectra, Atmos. Chem. Phys., 16(20), 13359–13378, doi:10.5194/acp-16-13359-2016, 2016.

Broadley, S. L., Murray, B. J., Herbert, R. J., Atkinson, J. D., Dobbie, S., Malkin, T. L., Condliffe, E. and Neve, L.: Immersion mode heterogeneous ice nucleation by an illite rich powder representative of atmospheric mineral dust, Atmos. Chem. Phys., 12(1), 287–307, doi:10.5194/acp-12-287-2012, 2012.

Cantrell, W. and Heymsfield, A.: Production of Ice in Tropospheric Clouds: A Review, Bull. Am. Meteorol. Soc., 86(6), 795–807, doi:10.1175/BAMS-86-6-795, 2005.

DeMott, P. J., Prenni, A. J., McMeeking, G. R., Sullivan, R. C., Petters, M. D., Tobo, Y., Niemand, M., Möhler, O., Snider, J. R., Wang, Z. and Kreidenweis, S. M.: Integrating laboratory and field data to quantify the immersion freezing ice nucleation activity of mineral dust particles, Atmos. Chem. Phys., 15(1), 393–409, doi:10.5194/acp-15-393-2015, 2015.

Murray, B. J., O'Sullivan, D., Atkinson, J. D. and Webb, M. E.: Ice nucleation by particles immersed in supercooled cloud droplets., Chem. Soc. Rev., 41(19), 6519–54, doi:10.1039/c2cs35200a, 2012.

Niedermeier, D., Shaw, R. A., Hartmann, S., Wex, H., Clauss, T., Voigtländer, J. and Stratmann, F.: Heterogeneous ice nucleation: Exploring the transition from stochastic to singular freezing behavior, Atmos. Chem. Phys., 11(16), 8767–8775, doi:10.5194/acp-11-8767-2011, 2011.

Polen, M., Lawlis, E. and Sullivan, R. C.: The unstable ice nucleation properties of Snomax® bacterial particles, J. Geophys. Res. Atmos., 121(19), 11,666-11,678, doi:10.1002/2016JD025251, 2016.

Welti, a., Lüönd, F., Kanji, Z. a., Stetzer, O. and Lohmann, U.: Time dependence of immersion freezing: an experimental study on size selected kaolinite particles, Atmos. Chem. Phys., 12(20), 9893–9907, doi:10.5194/acp-12-9893-2012, 2012.

Wex, H., Augustin-Bauditz, S., Boose, Y., Budke, C., Curtius, J., Diehl, K., Dreyer, A., Frank, F., Hartmann, S., Hiranuma, N., Jantsch, E., Kanji, Z. A., Kiselev, A., Koop, T., Möhler, O., Niedermeier, D., Nillius, B., Rösch, M., Rose, D., Schmidt, C., Steinke, I. and Stratmann, F.: Intercomparing different devices for the investigation of ice nucleating particles using Snomax® as test substance, Atmos. Chem. Phys., 15(3), 1463–1485, doi:10.5194/acp-15-1463-2015, 2015.

Wright, T. P. and Petters, M. D.: The role of time in heterogeneous freezing nucleation, J.

Geophys. Res. Atmos., 118(9), 3731–3743, doi:10.1002/jgrd.50365, 2013.

Zobrist, B., Koop, T., Luo, B. P., Marcolli, C. and Peter, T.: Heterogeneous ice nucleation rate coefficient of water droplets coated by a nonadecanol monolayer, J. Phys. Chem. C, 111(1), 2149–2155, doi:10.1021/jp066080w, 2007.

Response to Referee 2

This is an interesting paper where the authors apply a previously developed model to multiple component ice nucleating systems. They study ice nucleation by snowmax (which has multiple types of sites) and a mixture of snowmax plus nx illite. They con- clude that when mixed with illite the snowmax behaves much like pure snowax, which is important because this was not necessarily known (I mention this again below and I think the motivation for this study needs to be made clearer). The manuscript is rea- sonably well written, but I think the authors should try to be more concise wherever possible. In particular, use of scientific notation would make the manuscript far eas-ier to follow and must be corrected. In addition, I would like to see some discussion over the advantages/disadvantages of this method over singular descriptions. I support publication in ACP once the following specific points have been addressed.

We thank the reviewer for their comments and critiques. They have certainly helped us improve the message of our manuscript and clarified important aspects of the text. The referee's original comments are in italics, and our response follows.

P2., lines 18. How does 'analysis of ice crystal/precipitation sample residues' show that 'droplet freezing can occur at temperatures higher than -12 °C'?

In the cited studies, melted precipitation particles were collected and analyzed offline. The analysis typically involved forming droplets from the liquid sample and evaluating their freezing temperatures. Freezing at high temperatures (higher than -12 °C) was observed. We have reworded the text in the revised manuscript to make it more clear how the analysis works. The text now reads (Page/line 2/18-20):

"laboratory studies in which melted precipitation samples were refrozen (Christner et al., 2008; Petters and Wright, 2015; Vali, 1971, 1996) have shown that freezing can occur at temperatures higher than -12 °C."

P2, ln 20-25. 'higher than -12 C. This is a temperature range no investigated mineral samples of atmospherically relevant particle sizes (or other atmospherically relevant non-biological particles) can induce freezing in'. This is incorrect, there is a finite possibility of nucleation at these warmer temperatures. If the authors are referring to a situation in a cloud, are they sure about this? Very rare ice nucleation events seem to lead to secondary ice production, so is conceivable that mineral dust could account for a lot of very warm freezing.

The reviewer makes an excellent point. There is a finite probability of nucleating at higher temperatures given enough surface area of the material or given enough time (stochastic element of ice nucleation). We have reworded the statement to state that no investigated mineral samples of atmospheric relevant particle sizes have shown detectable ice nucleating activity in this temperature range. In this revised statement, no assumptions of zero probability of freezing are made but the practical finding of mineral dust's activity at these temperatures is highlighted. The revised manuscript now reads (Page/line 2/20-22):

"This is a temperature range no investigated mineral samples of atmospherically relevant particle sizes (or other atmospherically relevant non-biological particles) have exhibited detectable ice nucleating activity in (Atkinson et al., 2013; Cantrell and Heymsfield, 2005; DeMott et al., 2015; Murray et al., 2012)."

P3. In the main para in which the study is justified, some of the concepts introduced by O'Sullivan et al. [2016] should be mentioned. One reason why it is important to study dust-bio INPs is that it is not obvious that the proteins responsible for ice nucleation should retain their ice nucleating ability when adsorbed onto other materials. There is a literature on the subject of binding of proteins to clay surfaces and examples of where the function of the protein is impaired when adsorbed.

The study of O'Sullivan et al. (2016) is extremely relevant to the research questions posed in this manuscript. Text has been added to the intro to describe the contribution of that study on Page/line 3/9-11:

"O'Sullivan et al. (2016) found that ice nucleating proteins from soil can bind onto kaolinite and retain their HIN activity, thus creating an enhanced ice nucleating surface on the dust particle."

The findings of that study will also be discussed and compared with the findings here in the discussion part of the manuscript (see response to final comment below).

Intro. Some discussion of competing models would be useful. For example, how does this approach contrast with that of Knopf and Alpert [2013]. This is a very different approach, what are the strengths and weaknesses? For example, it could be argued that these multiple component models are not based on a physical model since the distribution of sites is empirically fitted to data.

Also, what advantage does this approach have over the commonly applied ice active site density?

In our response to this comment we address the following referee comment as well:

Fig 2. If this data were plotted as nm (i.e. sites per unit mass) would the different curves fall on *top of one another? How does this data compare with literature data for snowmax? Also, does nm capture enough detail to reproduce these fraction frozen curves, or is nm flawed?*

There are two parts to addressing these well-posed questions. The first is regarding the comparison between the single component version of this model (developed fully in Beydoun et al. (2016)) and other existing models. Section 3.4 in Beydoun et al. (2016) was dedicated to comparing our model with other existing ones. The n_s framework received very special attention in that paper, with comparisons of data in n_s or n_m shown throughout the whole manuscript. We argued in that work that n_s/n_m treatments to freezing data can lead to inconsistencies whereby the retrieved values don't overlap for different particle in droplet concentrations. We dedicated some of the analysis to the Snomax system in Beydoun et al. (2016) though it was only for four material concentrations and an old sample of Snomax that exhibited differences in HIN activity to the sample focused on in our newer study (see Polen et al. (2016) on Snomax INP degradation over time and storage). In that analysis of n_m spectra of Snomax we did find issues with value overlap, particularly a decrease in n_m for the same temperature as the mass concertation of Snomax in the droplets is decreased.

For the Snomax sample focused on in this study, we had made sure that its HIN activity was consistent with what other groups had reported. A comparison of the n_m values for all material concentrations with other groups is plotted on the right hand side of Fig. 3 in Polen et al. (2016) where it can be seen that the n_m values retrieved from the frozen fractions on the CMU cold plate lie within the envelope of n_m values summarized in Wex et al. (2015). We made sure this is mentioned in the manuscript on Page 8/19-21:

"The Snomax sample in this study is the same as that analyzed by Polen et al. (2016) whose freezing spectrum was shown to be consistent with the compilation of Snomax cold plate droplet freezing measurements summarized by Wex et al. (2015)."

It is typically found that retrieved values of n_m are within the same order of each other, so when plotted on a log scale to capture the full temperature spectrum as is done in Wex et al. (2015) the inconsistencies in n_m practically disappear. This does suggest that the ice active site density approach is not as problematic with Snomax as it is with NX illite or MCC cellulose (see Beydoun et al. (2016)) but depending on the level of detail one is looking to capture, the n_m approach is unable to consistently resolve the frozen fractions equally to the approach presented in this manuscript. It should also be emphasized that n_s/n_m is a diagnostic tool and is not used in a predictive fashion. n_m values are usually retrieved from the frozen fraction curves induced by a particle type and there is no effort to formulate for example an active site density distribution that can be extrapolated to different particle surface areas. In that regard, it is quite different than any approach that attempts to associate a contact angle or a distribution of contact angles to a particle type and attempt to predict the freezing behavior induced by this particle type for different particle surface areas. As for the approach of Alpert and Knopf (2016), a good deal of discussion on the differences between that approach and ours was presented in Beydoun et al. (2016) (see end of Sec. 3.4 in the referenced paper) where we had stated that surface area variability is an alternative approach to explaining the broadening of the frozen fraction curves.

The second part of addressing this comment is regarding the extended multi-component model developed here and its comparison to other approaches. We should have mentioned in the original version of the manuscript that the multi-component approach can be carried out by any of the other models as well. Equation (4), which states that the freezing probability of a droplet containing a mixture of particle types is the product of the independent freezing probabilities, can be developed using other formulations. However, we are unable to speculate about whether alternative treatments can succeed at predicting the behavior of the illite-Snomax mixture in the same way our approach has. We carry out a very distinct mathematical procedure in the random sampling of contact angles that has made it possible to model the transition from narrow to wide frozen fraction curves and capture the levelling of frozen fractions at low Snomax concentrations (see $1\text{x}10^{-5}$ to $1\text{x}10^{-7}$ wt% concentrations of Snomax in Fig. 2). From a mathematical perspective, the multi-component soccer ball model presented in Augustin et al. (2016) has as many free parameters as the multi-component model we present and therefore may be able to predict the behavior our model does. However, saying to what extent it can succeed would be overly speculative. In Broadley et al. (2012) the assumption of independent freezing probabilities was made to analyze a mixture of kaolinite and illite NX. The assumption did succeed in that study to predict the freezing behavior of the mixture.

Edits and additions to the revised manuscript were made regarding the second part of the response on multi-component models as the first part on the single component version has already been treated extensively in Beydoun et al. (2016). On Page/line 10/21-22 we have added a reference to Broadley et al. (2012) to indicate that the assumption of independent non-freezing probabilities was made there as well:

"It is assumed that every freezing probability is independent of the other, an approach similar to that taken by Augustin-Bauditz et al. (2016) and Broadley et al. (2012) for a mixture of birch wash water pollen and illite NX, and a mixture of illite NX and kaolinite, respectively."

On Page/line 11/17-19, we have added a statement saying the multi-component approach can be taken by any model of HIN activity and used to tests its validity to mixtures:

"It should be mentioned that derivations of and expressions similar to Eqn. (5) can be carried out using any of the other existing HIN frameworks starting from the assumption of independent freezing probabilities induced by each component."

Fig 1. The label (4) is missing from the images of droplets.

Scenario 4's droplet schematic is the same as the schematic on the top right hand corner of Fig. 1a, showing droplets containing dust surfaces larger than their corresponding critical areas. The revised part of the figure caption referring to scenario (4) should clarify this:

"Scenario (4) represents the absence of any biological material (Top right corner of Fig. 1a) and the resultant temperature freezing spectrum is identical to the one for dust lying above its critical surface area shown in the bottom of Fig. 1a."

P6. Top paragraph. The multiple component stochastic model described in Herbert et al. [2014] and Broadley et al. [2012] should be mentioned here.

The use of the word “component” in our study is used to describe a type of ice nucleating particle. For example, illite NX is comprised of one component while Snomax is comprised of two components. A single component possesses a distribution of contact angles and thus would be considered a multi-component surface under the definitions in Broadley et al. (2012) and Herbert et al. (2014). We realize now that this can be a source of confusion but hope that the definition of an ice nucleating component can clarify this to the reader. An explicit definition of a “component” in this study has been added on Page/line 7/9-10:

“In this study, a single component is defined as a particle type which can be treated as having one distribution of HIN activity.”

P7 What are ‘nucleation critical temperatures’?

In the original manuscript ‘critical nucleation temperatures’ was meant to describe the freezing temperatures induced by a particle’s HIN activity. We have removed this term in the revised manuscript and merely use ‘freezing temperatures of droplets’ instead.

Throughout: The term ‘ice nuclei’ is used. I urge the authors to use the terminally set out in the Vali ACP 2015 definitions paper. ‘Ice nuclei’ is used in the context of classical theory in reference to the cluster of water molecules, not the particle on which the cluster is stabilised.

We agree with adopting the terminology suggested by Vali et al. In the revised manuscript we use the term ice nucleating particle (INP) to refer to the ice nucleating entities in Snomax and illite NX and no longer use the term ‘ice nucleant’.

P11. Ln 15. Homogeneous nucleation can occur at a much higher T than -38 C. It is not that helpful to consider homogeneous nucleation occurring with a ‘limit’.

The reviewer makes a very valid point. The homogenous nucleation temperature is a function of the droplet volume (Vali, 1996). The text has been changed to state that the droplets containing no particle material freeze well above their expected homogenous freezing temperature range. The text has been revised on Page/line 12/5-7:

“The latter process does not strictly proceed through homogenous ice nucleation and causes freezing to happen at a much higher temperature than the expected homogeneous freezing temperature range (Vali, 1996) of the 400-600 µm droplets used in this study.”

P12, ln 14-16. Use scientific notation. In fact, use it throughout.

Scientific notation is now used throughout the entire text.

P23. Ln 13-15. Why is this an atmospherically relevant system?

In the original manuscript we described the system of droplets containing a mixture of illite-NX and very small Snomax concentrations because if a mixture of these two components did exist in the atmosphere it would in mostly likelihood contain a much larger mass percentage of dust than biological material. However, this does not make the system atmospherically relevant in the strictest sense of it actually being found in the atmosphere. Therefore, we have removed this description in the revised text.

P26. In the discussion of whether the snowmax-illite system is a 'close proxy to real atmospheric bio-dust mixtures', O'Sullivan et al. [2016] should be discussed. They argue that in soil particles will adsorb ice nucleating macromolecules. Soil borne fungus can shed its ice nucleating proteins into water and these proteins apparently bind to clay particles. This represents a distinct scenario of bio-dust mixtures compared to mixing snowmax with illte.

These are excellent points. We have added the findings of O'Sullivan et al. (2016) to the last paragraph of the discussion. We think this addition complements our findings quite nicely and motivates future work for investigating bio-dust mixtures. The text has been revised on Page/line 24/23-24 and Page 25/1-10:

"Regarding the motivating hypothesis that small biological particles can enhance the freezing capabilities of mineral dust surfaces, it can be concluded that the effectiveness of Snomax enhancing illite or any mineral dust surface is limited by its ability to partition itself and thus allow its HIN activity to be manifested externally. Hypothetical scenario 3, that was introduced in Section 1.2 and depicted in Fig. 1b, in which the limited amount of biological material does not distribute itself among all droplets, therefore closely resembles the bio-dust mixture proxy examined here. This is in contrast to the more effective behavior of soil borne fungus investigated in O'Sullivan et al. (2016). The biological macromolecules in that study were shown to bind onto clay and thus partition themselves among the dust surfaces, thereby distributing their HIN activity externally. This difference between Snomax and soil borne fungus should be kept in mind when considering the relevance of dust-bio mixtures in the atmosphere, and future work can attempt to quantify whether the ability of soil born fungus to adsorb unto clay surfaces can render it a stronger INP than Snomax despite its weaker HIN activity."

References

Alpert, P. A. and Knopf, D. A.: Analysis of isothermal and cooling-rate-dependent immersion freezing by a unifying stochastic ice nucleation model, Atmos. Chem. Phys., 16(4), 2083–2107, doi:10.5194/acp-16-2083-2016, 2016.

Beydoun, H., Polen, M. and Sullivan, R. C.: Effect of particle surface area on ice active site densities retrieved from droplet freezing spectra, Atmos. Chem. Phys., 16(20), 13359–13378, doi:10.5194/acp-16-13359-2016, 2016.

Broadley, S. L., Murray, B. J., Herbert, R. J., Atkinson, J. D., Dobbie, S., Malkin, T. L., Condliffe, E. and Neve, L.: Immersion mode heterogeneous ice nucleation by an illite rich powder representative of atmospheric mineral dust, Atmos. Chem. Phys., 12, 287–307, doi:10.5194/acp-12-287-2012, 2012.

Herbert, R. J., Murray, B. J., Whale, T. F., Dobbie, S. J. and Atkinson, J. D.: Representing time-dependent freezing behaviour in immersion mode ice nucleation, Atmos. Chem. Phys., 14(16), 8501–8520, 2014.

O'Sullivan, D., Murray, B. J., Ross, J. F. and Webb, M. E.: The adsorption of fungal ice-nucleating proteins on mineral dusts : a terrestrial reservoir of atmospheric ice-nucleating particles, Atmos. Chem. Phys., 16, 7879–7887, doi:10.5194/acp-16-7879-2016, 2016.

Polen, M., Lawlis, E. and Sullivan, R. C.: The unstable ice nucleation properties of Snomax® bacterial particles, J. Geophys. Res. Atmos., doi:10.1002/2016JD025251, 2016.

Vali, G.: Ice Nucleation - a Review, PoLAR, 271–279, 1996.

Wex, H., Augustin-Bauditz, S., Boose, Y., Budke, C., Curtius, J., Diehl, K., Dreyer, a., Frank, F., Hartmann, S., Hiranuma, N., Jantsch, E., Kanji, Z. a., Kiselev, a., Koop, T., Möhler, O., Niedermeier, D., Nillius, B., Rösch, M., Rose, D., Schmidt, C., Steinke, I. and Stratmann, F.: Intercomparing different devices for the investigation of ice nucleating particles using Snomax® as test substance, Atmos. Chem. Phys., 15(3), 1463–1485, doi:10.5194/acp-15-1463-2015, 2015.

A new multi-component heterogeneous ice nucleation model and its application to Snomax bacterial particles and a Snomax-illte mineral particle mixture

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Abstract

Some biological particles, such as Snomax, are very active ice nucleating particles, inducing heterogeneous freezing in supercooled water at temperatures above -15 °C and up to -2 °C. Despite their exceptional freezing abilities, large uncertainties remain regarding the atmospheric abundance of biological ice nucleating particles, and their contribution to atmospheric ice nucleation. It has been suggested that small biological ice nucleating macromolecules or fragments can be carried on the surfaces of dust and other atmospheric particles. This could combine the atmospheric abundance of dust particles with the ice nucleating strength of biological material to create strongly enhanced and abundant ice nucleating surfaces in the atmosphere, with significant implications for the budget and distribution of atmospheric ice nucleating particles, and their consequent effects on cloud microphysics and mixed-phase clouds.

The new critical surface area "$\bar{g}$" framework that was developed by Beydoun et al. (2016), is extended to produce a heterogeneous ice nucleation mixing model that can predict the freezing behavior of multi-component particle surfaces immersed in droplets. The model successfully predicts the immersion freezing properties of droplets containing Snomax bacterial particles across a mass concentration range of seven orders of magnitude, by treating Snomax as comprised of two distinct distributions of heterogeneous ice nucleating activity. Furthermore, the model successfully predicts the immersion freezing behavior of a low concentration mixture of Snomax and illite mineral particles, a proxy for the biological-dust mixtures

observed in atmospheric aerosols. It is shown that even at very low Snomax concentrations in the mixture, droplet freezing at higher temperatures is still determined solely by the second less active and more abundant distribution of heterogeneous ice nucleating activity of Snomax, while freezing at lower temperatures is determined solely by the heterogeneous ice nucleating activity of pure illite. This demonstrates that in this proxy system, biological ice nucleating particles do not compromise their ice nucleating activity upon mixing with dust and no new range of intermediary freezing temperatures associated with the mixture of ice nucleating particles of differing activities is produced. The study is the first to directly examine the freezing behavior of a mixture of Snomax and illite and presents the first multi-component ice nucleation model experimentally evaluated using a wide range of ice nucleating particle concentration mixtures in droplets.

1 Introduction

The potential role certain ice nucleating biological particles may play in cloud physics, meteorology, and global climate has been an active area of research for decades (Ariya et al., 2009; DeMott and Prenni, 2010; Franc and Demott, 1998; Möhler et al., 2007; Morris et al., 2004; Schnell and Vali, 1976). Biological particles such as *Pseudomonas syringae* bacteria can induce freezing in supercooled water at temperatures as high as -2 °C (Hartmann et al., 2012; Polen et al., 2016; Wex et al., 2015). While mineral dust particles are the leading candidate for the most abundant ice nucleating particles (INPs) in the atmosphere (Hoose et al., 2008; Murray et al., 2012), lidar and radar measurements (Bühl et al., 2013) as well as laboratory studies in which melted precipitation samples were refrozen (Christner et al., 2008; Petters and Wright, 2015; Vali, 1971, 1996) have shown that freezing can occur at temperatures higher than -12 °C. This is a temperature range no investigated mineral samples of atmospherically relevant particle sizes (or other atmospherically relevant non-biological particles) have exhibited detectable ice nucleating activity in (Atkinson et al., 2013; Cantrell and Heymsfield, 2005; DeMott et al., 2015; Murray et al., 2012). Furthermore, *in situ* chemical analysis of

Deleted: analysis of ice crystal/

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supercooled cloud drops and ice crystal residues found biological particle material contained within (Creamean et al., 2013; Kamphus et al., 2010; Pratt et al., 2009). All this evidence points to a potentially important role of biological particles in atmospheric ice nucleation, however significant constraints are still needed to properly account for its contribution to the total atmospheric INP burden and distribution (Möhler et al., 2007).

Schnell and Vali (1976) hypothesized that mineral dust particles can act as inert carriers of biological INPs. Their hypothesis keeps gaining support with early and more recent findings suggesting that biological particles release small ice nucleating macromolecules or fragments (Fröhlich-Nowoisky et al., 2015; Hiranuma et al., 2015b; Wolber et al., 1986) that can be easily lofted and transported on mineral dust surfaces (Augustin-Bauditz et al., 2016; O′Sullivan et al., 2015; Pratt et al., 2009). O'Sullivan et al. (2016) found that ice nucleating proteins from soil can bind onto kaolinite and retain their HIN activity, thus creating an enhanced ice nucleating surface on the dust particle. Despite all this emerging evidence of the existence of a heterogeneous ice nucleating (HIN) bio-dust atmospheric particle mixture, research on quantifying the freezing properties of controlled bio-dust mixtures in the laboratory remains sparse. Recently, Augustin-Bauditz et al. (2016) investigated the mixing state and HIN activity of illite NX mineral dust particles mixed with birch pollen wash water using the Leipzig Aerosol Cloud Interaction Simulator (LACIS). They found that when birch pollen existed on the mineral particle surface, the freezing temperature was determined solely by the biological material. This was concluded from their ability to model the freezing behavior of the mixed experiment using parameters derived from experiments on the individual components. While a seemingly obvious result, it is a worthwhile endeavor to investigate whether a mixture of particle components can produce a new intermediary range of freezing temperatures that isn't observed when each of the INP species is present individually. This helps to determine if the chemical mixing state – the extent to which different chemical components are mixed at the individual particle level – can modify each individual component's distribution of HIN activity, or if the total activity is just a linear addition of that of the individual components.

These mixed particle systems also provide a valuable opportunity to evaluate the ability of HIN models such as our recent $\bar{g}$ framework to predict the freezing properties of mixtures using the properties obtained from the pure components.

1.1 The influence of ice nucleating particle mixing state on droplet freezing temperatures

In Beydoun et al. (2016) the concepts of internal variability and external variability in HIN activity were defined and shown to have a dramatic impact on the temperature range a droplet population freezes in. If the same distribution of HIN activity is contained within each particle in an aerosol population (the internal variability), then the range of temperatures over which these particles cause freezing in the droplets they are contained in would be significantly different than if that same distribution was distributed externally (the external variability). Figure 1a illustrates the concepts of internal versus external variability. In the top right-hand panel of Fig. 1a each droplet contains a particle surface with a color gradient representing the distribution of HIN activity. Note that each particle contains the same distribution of HIN – demonstrating full internal variability – and therefore the range of temperatures over which droplets freeze is relatively narrow, as depicted in the bottom panel. As the surface area of the particle surface in each droplet decreases, the HIN distribution becomes externally variable, meaning that each droplet now contains a different distribution of HIN activity, which is shown with the different colors the particle surfaces are illustrated with in the top left hand panel of Fig. 1a. The reduced surface area per droplet leads to a reduction in the freezing temperatures while the increased external variability leads to a broader temperature range over which droplets freeze relative to the larger surface area case where the variability in HIN activity between each droplet was eliminated.

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Deleted: The droplet freezing temperature spectrum is predicted using the g framework we developed in Beydoun et al. (2016) and expand upon here to predict multi-component particle mixtures. The distribution of HIN activity for Snomax and illite NX was taken from that determined in our prior report.

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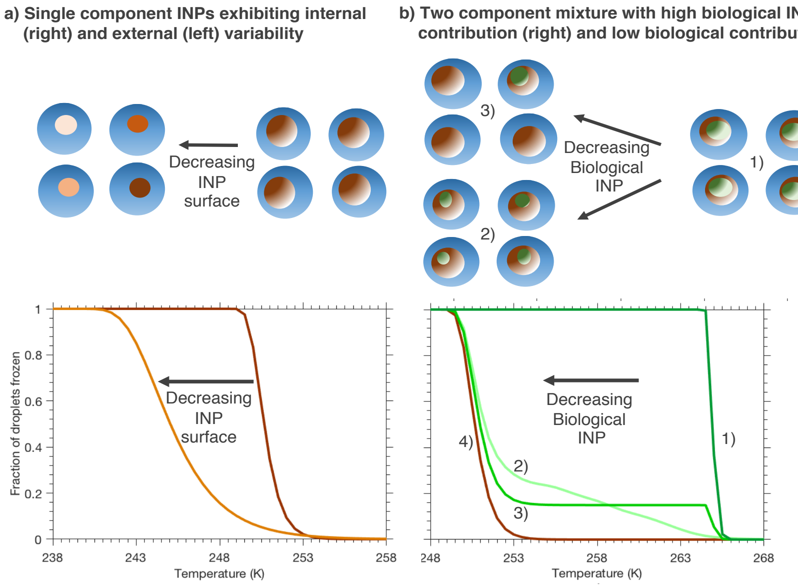

a) Single component INPs exhibiting internal (right) and external (left) variability

b) Two component mixture with high biological INP contribution (right) and low biological contribution (left)

a) Top: Right: Droplets containing particle surfaces larger than the component's critical surface area exhibiting internal variability (color gradient) in HIN activity. Left: Droplets contain particle surfaces smaller than the component's critical surface area and exhibit external variability in HIN depicted by the different colors. **a) Bottom:** Decreasing surface area leads to higher external variability in HIN activity and thus a wider range over which droplets freeze. **b) Top:** Right: Droplets contain a mixture of mineral dust (brown) and biological (green) particle surfaces, both of which are larger than their component's critical surface area. Left: Two hypothetical scenarios of reduced biological particle surface areas are depicted as the concentration of biological INP is reduced. In (2) the biological material distributes itself equally among the droplets and thus remains internally mixed while in (3) the biological material does not further partition itself further and becomes externally mixed. Scenario (4) represents the absence of any biological material (Top right corner of Fig. 1a) and the resultant temperature freezing spectrum is identical to the one for dust lying above its critical surface area shown in the bottom of Fig. 1a. **b) Bottom:** The predicted impact of the INP concentration and mixing state of the dust-bio mixture on the resultant freezing spectrum of droplets containing the particle mixture.

To place the concepts of internal versus external variability in the context of cloud evolution one can consider the example study conducted by Ervens and Feingold (2012) in which different HIN treatments were compared in a cloud air parcel model. A close examination of the impact of variability in HIN activity was performed, whereby different HIN schemes – that inherently make assumptions about whether HIN activity is externally variable or internally variable – were compared. For example the alpha-PDF scheme (Welti et al., 2012) assumes total external variability, while an internally mixed soccer ball model (Niedermeier et al., 2011) assumes total internal variability. The different schemes resulted in a difference of a factor of 3 in the ice crystal concentration following cloud glaciation. The ice water content distribution with height was also different, whereby the alpha-PDF scheme resulted in higher variability in ice water content versus altitude due to it inducing droplet freezing over a wider temperature and thus altitude range.

While the particle chemical component mixing state and HIN external versus internal variability are not one in the same, the two properties are certainly relatable. If an aerosol particle population is chemically diverse and this diversity is externally distributed, then the freezing capabilities of the entire particle population would need to be defined by many different distributions of HIN activity; each particle would have a different distribution of ice nucleation ability. This becomes more significant with components exhibiting very different HIN properties, such as biological and mineral particles. A completely internally mixed aerosol particle population on the other hand can imply total internal variability in HIN activity; each particle has the same composition and therefore the same ice nucleation ability. In considering illite NX for example, this widely used proxy for atmospheric mineral dust is composed of illite clay along with smaller amounts of kaolinite, quartz, calcite, and feldspars minerals (Hiranuma et al., 2015a). Despite this chemically diverse profile, one distribution of HIN activity $\bar{g}$ was sufficient to accurately describe the freezing behavior of illite NX over many orders of magnitude of particle surface area and consequently a wide freezing temperature range from -20 °C down to -35 °C (Beydoun et al., 2016).

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Returning to the bio-dust mixed particles, it is likely that in a totally internally variable mixture the HIN activity is simply determined by the biological particles given their much stronger HIN activity that induces freezing > -10 °C. The top right-hand panel of Fig. 1b illustrates this, whereby each droplet contains both large biological and mineral dust surfaces and the consequent freezing behavior of the droplets is identical to the case of only biological surfaces present. What is less clear is what happens in the limit of very low biological particle concentrations and relatively high mineral dust particle concentrations. Does the system then behave as an externally mixed system in which the droplets containing biological particles freeze according to the HIN activity of the biological surfaces while the rest of the droplets freeze as dictated by the dust? Or do the low biological particle concentrations generate a new range of freezing temperatures lying between the freezing temperatures of the two individual and separated particle components? These two scenarios are depicted in the left-hand panel of Fig. 1b along with their hypothesized freezing behaviors in the frozen fraction spectrum underneath.

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This paper extends the heterogeneous ice nucleation model presented in Beydoun et al. (2016) to droplet systems comprised of different HIN components. In this study, a single component is defined as a particle type which can be treated as having one distribution of HIN activity. The extended treatment is then used to model the freezing behavior of Snomax, a freeze-dried powder manufactured from nonviable *Pseudomonas syringae* bacteria, immersed in droplets and analyzed on a cold plate. Snomax's ice nucleation properties are attributed to large protein aggregates, and it is often used as a proxy for atmospheric biological INP (Pandey et al., 2016; Polen et al., 2016; Wex et al., 2015). It is argued that Snomax is itself a mixture of two components of INP's and necessitates the extended mixing model to properly describe its entire freezing spectrum. The notion that Snomax is a mixture of INPs of different activities is consistent with the hypothesis that the substance is composed of protein aggregates of different sizes and abundances, particularly the Type I and Type III protein aggregates, that exhibit different freezing temperatures (Hartmann et al., 2012; Turner et al., 1990; Yankofsky et al., 1981). The extended model is then applied to a mixture of illite and Snomax at varying concentrations of the latter to examine how well these two different regimes of HIN activity remain indistinguishable when present in the same droplet, and what the consequent atmospheric implications are.

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2 Experimental and computational methods

2.1 Experimental ice nucleation measurements

The cold plate assay used to retrieve the freezing spectra for this study is similar to that described by Polen et al. (2016). Some upgrades to the system have taken place, such as the use of a two-stage thermoelectric cooling (TEC) system comprised of an enclosed air-cooled three-stage cascade cold plate (TECA, AHP-1200CAS) mounted below a single-stage TEC module (TETech, VT-127-1.0-1.3-71) and the custom-built cold plate droplet freezing chamber. The cascade cold plate acts as the heat sink while the one-stage TEC modulates the temperature of the cold plate chamber where the droplets reside. An aluminum dish is placed in the chamber inside which a hydrophobic glass coverslip is immersed in squalene oil. An electronic 0.1 µl pipette (Sartorius eLINE) has also been recently introduced to produce droplets of more consistent sizes. This reduces the total particle surface area variability between individual droplets.

A solution of ultapure Milli-Q water and the particle material being investigated was prepared, from which 40-60 0.1 µl droplets were generated (resulting in a 0.02 resolution in the retrieved frozen fraction) using the electronic pipette and placed on the coverslip immersed in the oil. A cooling rate of 1 K/min was used and images of the droplet array were collected at 1 Hz using an optical microscope and CMOS camera. The images were then used to deduce the fraction of droplets frozen at each temperature; frozen droplets appear black. Each freezing experiment was repeated at least twice to confirm that the independently retrieved frozen fractions fall within 1 K (the temperature measurement uncertainty) of each other for each replicate experiment and that the total number of particle/droplet pairings being examined exceeds 100. The samples used in this study were commercial freeze-dried Snomax powder (York International) and illite NX (Arginotec, NX nanopowder) (Hiranuma et al., 2015a). The illite sample is identical to that used in Beydoun et al. (2016) and therefore the HIN properties derived there are reused for the analysis here. The Snomax sample in this study is the same as that analyzed by Polen et al. (2016) whose freezing spectrum was

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shown to be consistent with the compilation of Snomax cold plate droplet freezing measurements summarized by Wex et al. (2015). Suspensions from which the droplets were generated were prepared by mixing a known mass of the sample (Snomax or illite) with a known volume of ultrapure Milli-Q water to form a suspension with a specific weight percentage of the sample in water. The droplets containing a mixture of Snomax and illite were generated from a suspension formed by creating a 1:1 mixture from the pure illite and pure Snomax suspensions.

2.2 The critical particle surface area hypothesis, g, and $\overline{g}$

In Beydoun et al. (2016) we started from classical nucleation theory to formulate a framework of heterogeneous ice nucleation which states that any given particle surface can be assigned a distribution of HIN activity, g. g is a continuous normal distribution of contact angles, θ (used as a proxy for the HIN activity at the particle surface-water interface), and determines the probability that a particle induces freezing in a droplet, P_f, at a temperature, T, via:

$$P_f(T) = 1 - \exp\left(-tA\int_0^{\pi} g(\theta)J(\theta,T)d\theta\right) \qquad (1)$$

where t is the time the droplet spent at the temperature T, A is the surface area of the particle, and $J(\theta, T)$ is the heterogeneous ice nucleation rate per unit surface area per unit time as defined in Zobrist et al. (2007). The freezing probability can also be written for a droplet undergoing a constant cooling rate $\dot{T}$ as:

$$P_f = 1 - \exp\left(-\frac{A}{\dot{T}}\int_{T_i}^{T_f}\int_0^{\pi} g(\theta)J(\theta,T)d\theta dT\right) \qquad (2)$$

For a large ensemble of droplets containing identical particle surfaces, the freezing probability is equal to the fraction of droplets frozen, F.

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We hypothesized that an individual particle type possesses a critical surface area, above which one normal distribution of HIN activity $\bar{g}$ can accurately describe the freezing probability of a droplet containing any particle of that type. Therefore, the freezing probability of a droplet containing a particle with a surface area larger than this critical area can be evaluated using Eqns. (1) or (2) with $\bar{g}$ in place of g, and the fraction of droplets frozen, F, of a large ensemble of these droplets N is equal to the freezing probability of each droplet, P_f. On the other hand, when a particle possesses a surface area below that type's critical surface area, a random sampling of contact angles to generate a discrete distribution of HIN activity g^* is required for each particle surface in the particle population. In this case the frozen fraction of a large ensemble of droplets, F, is the arithmetic mean of the individual droplet freezing probabilities and can be evaluated using:

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$$F = \frac{1}{N}\sum_{i=1}^{N} P_{f,i}\left(g^*{}_i(n_{draws})\right) \qquad (3)$$

where $P_{f,i}$ is the freezing probability of droplet i and can be evaluated using Eqns. (1) and (2) with g^* used in place of g and n_{draws} is the number of contact angle draws made from $\bar{g}$. The effect of decreasing an INP's total surface area below its critical surface area threshold on the distribution of HIN activity contained in each droplet is illustrated in Fig. 1 by the extensive broadening of the droplet freezing temperature curve when below the critical area. Above the critical area the diversity of HIN activity is essentially eliminated across the particle population; the large available surface area guarantees that the full distribution of HIN activity is contained in each droplet and described by $\bar{g}$. The creation of the g^* distribution by taking n_{draws} from $\bar{g}$ accounts for this increased diversity in HIN activity when below the critical area threshold.

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2.3 Mixing model

The mixing model extends the HIN framework reviewed above to account for multiple distributions of ice nucleation activity presented by multiple INP types inside a single droplet, as well as the contribution from homogenous ice nucleation. It is assumed that every freezing probability is independent of the other, an approach similar to that taken by Augustin-Bauditz et al. (2016) and Broadley et al. (2012) for a mixture of birch wash water pollen and illite NX, and a mixture of illite NX and kaolinite, respectively. The freezing probability of an individual droplet containing some mixture of components $n_c + n_{sc}$, where n_c is the number of components with surface areas greater than their corresponding critical surface areas and n_{sc} is the number of components with surface areas lower than their corresponding critical surface areas, is:

$$P_f = 1 - P_{uf,hom} \prod_{k=1}^{n_c} P_{uf,c,k}(\bar{g}_k, A_k) \prod_{j=1}^{n_{sc}} P_{uf,sc,j}({g^*}_j, A_j) \qquad (4)$$

where $P_{uf,hom}$ is the probability freezing does not occur due to homogenous nucleation, $P_{uf,c,k}$ is the probability freezing does not occur due to component *k* possessing a surface area A_k lying above this component's critical area and thus can be evaluated with $\bar{g}_k$. $P_{uf,sc,j}$ is the probability freezing does not occur due to component *j* possessing a surface area A_j lying below this component's critical surface area and thus requires random sampling from $\bar{g}_j$ to generate g_j^* for evaluation.

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For a large ensemble of *N* droplets, the frozen fraction *F* is the mean of the individual droplet freezing probabilities. This yields the following expression for the frozen fraction of droplets*:*

$$F = 1 - \frac{1}{N} \sum_{i=1}^{N} \left[\exp\left(-\frac{1}{\dot{T}} \int_{T_i}^{T_f} \left(J_{hom} V + \sum_{k=1}^{n_c} A_k \int_0^{\pi} \bar{g}_k(\theta) J(\theta, T)\, d\theta \right.\right.\right.$$

$$\left.\left.\left. + \sum_{j=1}^{n_{sc}} A_j \int_0^{\pi} {g^*}_{i,j}(\bar{g}_j, n_{draws,j}) J(\theta, T) d\theta \right) dT \right) \right] \qquad (5)$$

where $\dot{T}$ is the cooling rate, T_i and T_f are the initial and final temperatures in a cooling experiment, J_{hom} is the homogenous nucleation rate, *V* is the droplet volume, and $n_{draws,j}$ is the number of times random sampling takes place from $\bar{g}_j$. An additional subscript *i* is added to g^* to indicate that the sub-critical area distribution of contact angles (θ) for a component k will vary between droplets. It should be mentioned that derivations of and expressions similar to Eq. (5) can be carried out for any of other existing HIN frameworks starting from the assumption of independent freezing probabilities induced by each component.

For the purposes of the analysis of the experiments presented in this paper, Eqn. (5) can be reduced to describe one component exhibiting behavior above its critical area, one component exhibiting behavior below its critical area, and the background HIN distribution of impurities in the species being examined or the milli-Q water itself. The latter process does not strictly proceed through homogenous ice nucleation and causes freezing to happen at a much higher temperature than the expected homogeneous freezing temperature range of the 400-600 μm droplets used in this study. Eqn. (5) is thus reduced to:

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$$F = 1 - \frac{1}{N}\sum_{i=1}^{N}\left[\exp\left(-\frac{1}{\dot{T}}\int_{T_i}^{T_f}\left(A_1\int_0^{\pi} g^*{}_{i,1}(\bar{g}_j, n_{draws,j})J(\theta,T)d\theta + A_2\int_0^{\pi}\bar{g}_2(\theta)J(\theta,T)\,d\theta + \int_0^{\pi} G_{bg}(\theta)J(\theta,T)d\theta\right)dT\right)\right] \quad (6)$$

It should be noted that the distribution of ice nucleating activity of the background impurities has been lumped with its implied particle surface area into one term G_{bg}. This simplification serves the same purpose of simply accounting for droplet freezing that could be occurring due to background impurities. The contribution from this term becomes particularly important at low particle concentrations. G_{bg} is thus a normal distribution multiplied by a pre-factor making it a function of three independent parameters: the pre-factor (C), the mode (μ), and the standard deviation (σ).

3 Results and discussion

3.1 Snomax: Two distributions of heterogeneous ice nucleating activity

The HIN behavior of Snomax was investigated by varying the concentration of the prepared particle suspension to retrieve the full freezing temperature spectrum of the system. Similar droplet freezing measurements of Snomax have been conducted and analyzed before as summarized in Wex et al. (2015). However, the analysis presented here is unique due to the application of the newly developed critical area method we presented in Beydoun et al. (2016) and summarized above. Freezing spectra for Snomax mass concentrations of 1x10^{-1}, 5x10^{-2}, 3x10^{-2}, 1x10^{-2}, 5x10^{-3}, 1x10^{-3}, 5x10^{-4}, 1x10^{-4}, 5x10^{-5}, 1x10^{-5}, and 1x10^{-6} wt% were obtained and are plotted in Fig. 2a. Figure 2b extends Fig. 2a to show the freezing spectra corresponding to Snomax mass concentrations of 5x10^{-7} and 1x10^{-7} wt%.

For Snomax concentrations of 1x10^{-1} wt% through 5x10^{-4} wt% the temperatures over which droplets freeze gradually broadens and decreases as the concentration is decreased. This is consistent with the trend observed with the systems analyzed in Beydoun et al. (2016) in which the broadening is interpreted as particle surface areas becoming smaller than the species' critical area, which creates external variability in the HIN activity of the particle surface available in each droplet. The highest two concentrations (1x10^{-1} and 5x10^{-2} wt%) can be fit with the same g distribution. The solid lines in Fig. 2a indicate a single g fit. A least squares error approach is used to find the g distribution of θ (μ, σ) that best models the highest concentration freezing curve and this g is then reused to predict the second highest concentration freezing curve, following the procedure outlined in Beydoun et al. (2016). At concentrations lower than 5x10^{-2} wt% a single g fit fails at predicting the behavior of the freezing curves. The behavior is defined by the median freezing temperature at which 50% of the droplets freeze and the range of temperatures over which droplets freeze. A failed fit produces a freezing curve that is not within the 1 °C experimental temperature uncertainty of each experimental frozen fraction data point. The successful fitting of the frozen fraction curves at the two highest

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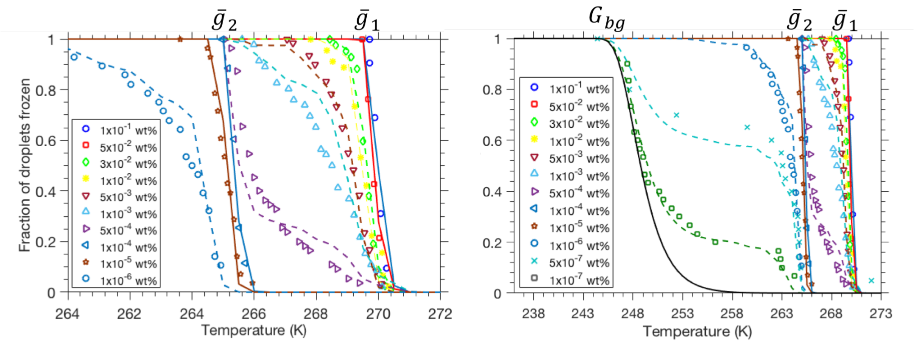

Figure 2. a) Frozen fraction temperature spectra for Snomax mass concentrations of 1x10^{-1}, 5x10^{-2}, 3x10^{-2}, 1x10^{-2}, 5x10^{-3}, 1x10^{-3}, 5x10^{-4}, 1x10^{-4}, 1x10^{-5}, and 1x10^{-6} wt% (symbols). Critical area fits using two distinct Snomax distributions of HIN activity (solid lines) are shown for some droplet freezing curves, while the rest of the freezing curves are modelled with the mixing model (dashed lines). **b)** All frozen fraction curves shown in a) in addition to the lowest Snomax concentration frozen fraction curves of 5x10^{-7} and 1x10^{-7} wt%. The lowest concentration freezing curves are modelled using random sampling by n_{draws} of the $\bar{g}_2$ distribution of HIN activity. The solid black line is the assumed frozen fraction curve induced by the background impurities in the droplets.

Snomax concentrations (1x10^{-1}and 5x10^{-2} wt%) using one g distribution suggests that the particle surface areas inside the droplets fall above the critical surface area threshold. Therefore, the g retrieved is $\bar{g}$. Conversely the emergence of the broadening trend in freezing temperatures with decreasing Snomax concentration starting with the 3x10^{-2} wt% frozen fraction curve suggests that particle surface areas inside each droplet lie below the critical area threshold and consequently exhibit external variability in their HIN activity. The broadening trend continues with decreasing particle concentration until the 1x10^{-4} wt% Snomax concentration, whereby a steep freezing curve with a narrow range of freezing temperatures re-emerges. The g_2 distribution that fits this low concentration freezing curve is a different Gaussian function than the g_1 distribution that fits the first two high concentration freezing curves (after accounting for the change in particle surface area). Furthermore, the g_2 distribution obtained from the 1x10^{-4} wt% freezing curve also fits the 1x10^{-5} wt% freezing curve before a new trend of frozen fraction curve broadening emerges starting with the 1x10^{-6} wt% concentration. Figure 1b expands the temperature range of the frozen fraction curves to show the unique trend of the very low concentration freezing curves (5x10^{-7}and 1x10^{-7} wt%). Only the initial part

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of these frozen fraction curves exhibits broadening and freezing at high temperatures beyond which there is an approximate plateau without further droplet freezing between 263 and 253 K. The plateau ends around a similar temperature range between 253 K and 248 K for both low particle concentrations and they converge around what is probably freezing due to background impurities in the water, the sample, or the hydrophobic coverslip. These background impurities will be discussed in more detail later in this section.

Based on these observations, it is hypothesized that Snomax can be modelled as having two distinct distributions of HIN activity, each possessing its own critical surface area threshold. Using the fits to the frozen fraction curves of the 1x10^{-1} and 5x10^{-2} wt% Snomax concentrations, the first distribution denoted $\bar{g}_1$ is a normal distribution with parameters $\mu_1 = 0.60$ and $\sigma_1 = 0.050$ (Eqn. (2)). Similarly, the fits to the frozen fraction curves of the 1x10^{-4} and 1x10^{-5} wt% Snomax concentrations give the second distribution of HIN activity denoted $\bar{g}_2$ with parameters $\mu_2 = 0.52$ and $\sigma_2 = 0.0001$. This can be viewed as a mathematical interpretation of Snomax's hypothesized Type I and Type III proteins, which are said to cause freezing at similar temperature regimes to where the first and second critical areas have been identified from the freezing spectra in Fig. 2 (Turner et al., 1990; Yankofsky et al., 1981). For this analysis it is assumed that all HIN components of Snomax have surface area densities of 1 m^2/g. While not based on an empirical value, the surface area density assumption is merely invoked since the model is set up such that freezing probabilities are evaluated as a function of surface area and not mass. The assumption does not affect the insight gained from the analysis. This results in 1x10^{-5} cm^2 and 1x10^{-8} cm^2 for the first and second critical surface areas thresholds, respectively, of the $\bar{g}_1$ and $\bar{g}_2$ distributions of HIN activity. Note that the $\bar{g}_1$ is responsible for the freezing observed at higher temperatures when higher Snomax concentrations are used.

The Snomax particle concentrations of 3x10^{-2} through 5x10^{-4} wt% that correspond to the frozen fraction curves between the two steep sets of frozen fraction curves are thus considered to possess surface areas between the critical area thresholds of the first and second distribution of Snomax INP's, whose HIN activity

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are described by $\bar{g}_1$ and $\bar{g}_2$. Modelling these freezing curves is thus the first application of the extended mixing model summarized in Eqn. (6) as there are two distinct and distinguishable types of INPs present in droplets containing Snomax particles. The g^* distributions describe the more-active $\bar{g}_1$ HIN type for which the Snomax concentrations are below its critical area threshold. The g^* distributions used in the first term inside the exponential in Eqn. (6) are retrieved by taking a number of random θ values, n_{draws}, from $\bar{g}_1$, whereby each frozen fraction curve will have a unique n_{draws} that produces a modelled frozen fraction curve closest to the measured curve. The random sampling process is repeated up to three times to ensure repeatability of the modelled frozen fraction curve. The optimization of n_{draws} is carried out manually as the range of n_{draws} which results in changes to the modelled frozen fraction curves is small enough such that a more comprehensive numerical optimization (e.g. a Monte Carlo simulation) was not found to be necessary. In the case of the mixture of two distributions of HIN activity in Snomax, A_1 is assumed equal to A_2 and is calculated from the Snomax concentration and the assumed surface area density. The last term corresponding to the HIN activity of background impurities can be safely neglected here as the freezing temperatures are much higher than temperatures at which background impurities typically cause freezing in this system (< 248 K). The consequent n_{draws} for each frozen fraction curve between the two Snomax critical areas are: 80 for the 3x10^{-2} wt%, 65 for 1x10^{-2} wt%, 40 for 5x10^{-3} wt%, 25 for 1x10^{-3} wt%, and 6 for 5x10^{-4} wt%. The modelled frozen fraction curves are plotted as dotted lines in Figs. 2a and 2b.

Modelling the frozen fraction curve for the lowest concentration frozen fraction curve lying between the two critical surface areas (5x10^{-4} wt% Snomax concentration) necessitated placing a lower contact angle limit when conducting random sampling. At a low number of n_{draws} it becomes likely that some g^* distributions become unrealistically active due to over sampling of lower contact angles and consequent normalization of the distribution as outlined in Beydoun et al. (2016). Therefore, a lower contact angle limit of θ = 0.220 radians was invoked for modelling this frozen fraction curve. It should also be mentioned that the choice of this lower contact angle limit is not arbitrary as it is based on a nucleating surface area analysis introduced

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in Beydoun et al. (2016) of the highest concentration frozen fraction curve (0.1 wt% Snomax) in which it was found that 0.220 radians approximated a critical contact angle below which contact angles do not comprise an appreciable surface area to contribute to nucleation. Therefore, if any θ smaller than 0.220 does not contribute to freezing at particle surface areas above the critical surface area threshold then it should not contribute to freezing for surface areas below it. It should be emphasized that this special invocation was only done for modelling one of the frozen fraction curves in the entire study. This last point is a recognized caveat of the model and improvements to the numerical technique to be more consistent across the entire range of n_{draws} is a subject of ongoing research.

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A similar analysis was performed to model the frozen fraction curves with surface areas below the second critical area threshold, which correspond to Snomax concentrations of $1\text{x}10^{-6}$, $5\text{x}10^{-7}$, and $1\text{x}10^{-7}$ wt%. In this case Eqn. (6) is used without the contribution from a component with a surface area larger than its own critical area threshold (the second term inside the exponential). Random sampling to produce the g^* distributions is done from $\bar{g}_2$, which was determined using the $1\text{x}10^{-4}$ and $1\text{x}10^{-5}$ wt% Snomax curves as described above. The HIN activity of background impurities has an important contribution for these low concentrations as can be seen in Fig. 2b by the fact that all three of these frozen fraction curves with surface areas below the second critical surface area threshold finish freezing along the same low temperature freezing line. This freezing line is assumed to be due to the HIN activity of the background impurities. It should be

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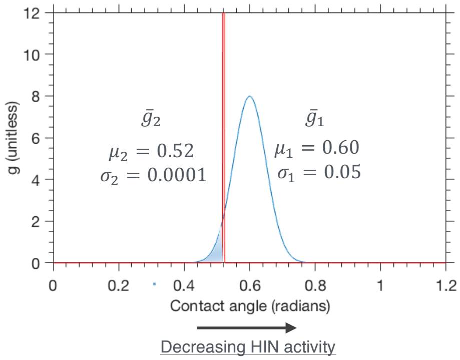

Figure 3. The two distributions of HIN activity, $\bar{g}_1$ (right, broad, in blue) and $\bar{g}_2$ (left, narrow, in red), retrieved from the Snomax droplet freezing spectra (Fig. 2).

noted however that this freezing line is not reproducible when retrieving the frozen fraction curve of droplets made up only of Milli-Q water, which freeze 5 K colder than this line. It was also not possible to reproduce this background freezing spectrum with a newly acquired batch of Snomax, whereby droplets containing particle material from the new batch froze at a lower temperature than the droplets containing particle material from the old batch. Therefore, it is likely that these impurities are associated with this particular Snomax sample, so their resultant frozen fraction curve will simply be added to the mixing model in the form of G_{bg}. G_{bg} is derived by extrapolating the low temperature part of the frozen fraction curve of the lowest Snomax concentration (solid blue line in Fig. 1b). The resultant parameters are $\mu_{bg} = 2.8$ and $\sigma_{bg} = 0.25$ and a pre-factor $C = 2.5\text{x}10^{-6}$. The consequent n_{draws} for each frozen fraction curve below the second Snomax critical area threshold are: 8000 for $1\text{x}10^{-6}$ wt%, 2800 for $5\text{x}10^{-7}$ wt%, and 750 for $1\text{x}10^{-7}$ wt%. The much higher n_{draws} values for this range of concentrations are due to the much narrower range of HIN activity that distribution $\bar{g}_2$ covers compared to distribution $\bar{g}_1$ (σ_2 is much smaller than σ_1, 0.0001 versus 0.05). A larger number of draws from $\bar{g}_2$ is therefore required to capture its HIN activity.

Figure 3 shows the two Snomax $\bar{g}$ distributions plotted alongside each other for comparison. The first striking contrast lies in the σ value for each distribution ($\sigma_1 = 0.05$ vs. $\sigma_2 = 0.0001$), which defines the range

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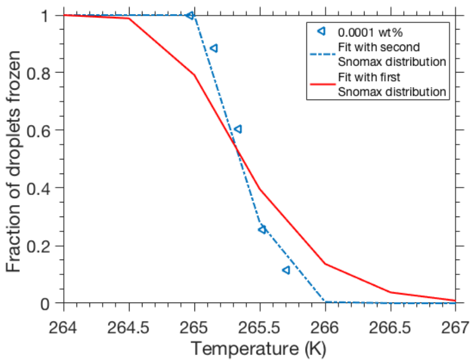

Figure 4. The 1x10^{-4} wt% Snomax frozen fraction curve (symbols), corresponding to the highest concentration freezing spectrum where freezing was exclusively determined by the $\bar{g}_2$ distribution of HIN activity. Freezing spectra fits are also included, one derived using the $\bar{g}_2$ distribution of HIN activity (dashed line), while the other is a hypothetical fit corresponding to the activity of the $\bar{g}_1$ distribution (solid line).

of contact angles a g distribution covers. Typically, a larger σ does lead to a broader range of freezing temperatures when the surface area is larger than its critical area. However, for the same σ, frozen fraction curves at higher temperatures are steeper (exhibit freezing over a narrower temperature range) than at lower temperatures, due to the strength of the range of HIN activity causing higher temperature freezing. The temperature range where freezing ensues is determined by the distribution of contact angles, which is governed by both μ and σ. While merely looking at the frozen fraction curves dictated exclusively by either $\bar{g}_1$ or $\bar{g}_2$ in Fig. 2 would hint at similar HIN activity contact angle breadth, it should be emphasized that the contact angle range for $\bar{g}_2$ must be narrower (smaller σ) and/or have a larger mode value, μ, due to it causing freezing at lower temperatures. In other words, if $\bar{g}_1$ was used to try and fit the frozen fraction curves associated with $\bar{g}_2$, the consequent fit would not only overpredict the freezing temperature but also the range of temperatures over which freezing would occur. Figure 4 shows a fit for the 1x10^{-4} wt% frozen fraction curve that uses $\bar{g}_1$ instead of $\bar{g}_2$. The required surface area input for this alternative fit of the 1x10^{-4} wt% frozen fraction curve is smaller by a factor of 10^{-8} than the surface area used for the 1x10^{-1} wt% frozen fraction curve, when it should only be smaller by a factor of 10^{-3} based on the differences in Snomax mass

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concentration of each curve. Furthermore, the range of temperatures over which freezing occurs using $\bar{g}_2$ is noticeably broader for the fit using $\bar{g}_1$, which supports the point made above.

A narrower σ for the $\bar{g}_2$ distribution is also reflected in the very abrupt transition in freezing behavior that droplets containing low Snomax concentrations undergo with decreasing concentration. As depicted in Fig. 2b, after a second Snomax critical area regime determined exclusively by $\bar{g}_2$ that covers 2 orders of magnitude in concentration (1x10^{-4} and 1x10^{-5} wt%) a sub-critical area regime begins (the broader 1x10^{-6} wt% frozen fraction curve) followed by a very quick transition (after only a factor of 5 concentration decrease) to a loss of Snomax HIN activity in at least 60% of the droplets (5x10^{-7} wt% frozen fraction curve). While frozen fraction curves lying between critical area 1 and critical area 2 covered two orders of magnitude in concentration before partial loss of HIN activity represented by $\bar{g}_1$, frozen fraction curves lying below critical area 2 covered only one order of magnitude. Furthermore, the continually dominant contribution from the contact angle range causing freezing at a high temperature of about 265 K to 263 K also points to a narrow second distribution of HIN activity. This can be seen in the transition between Snomax concentrations of 1x10^{-6} wt% to 5x10^{-7} wt% and 1x10^{-7} wt% whereby the two lowest concentration freezing curves exhibit most of their freezing at the higher temperatures (265 K-263 K) of the temperature range the second distribution of HIN contributes to (Fig. 2b). However, it should be noted that the existence of some broadness in the temperature over which droplets freeze throughout the regime falling below the second critical area does necessitate that $\bar{g}_2$ still has a finite σ, albeit a small one.

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The second notable feature in the contrast between the two Snomax distributions of HIN activity shown in Fig. 3 is that the mode of the less active $\bar{g}_2$ distribution ($\mu_2 = 0.52$) is actually at a smaller contact angle (i.e. more ice active) than that of the $\bar{g}_1$ distribution ($\mu_1 = 0.60$). This is not contradictory to the resultant freezing curves where the JNP's represented by $\bar{g}_1$ cause freezing at several degrees warmer than those of $\bar{g}_2$. The freezing temperatures are actually determined by the ascending tail of the first distribution for the

Deleted: ice nucleants represented by $\bar{g}_1$ freeze several degrees warmer than the $\bar{g}_2$ ice nucleants. Given the deterministic control of ice nucleation as temperature is decreased, the ice nucleant with the warmest critical temperature in the droplet determines its freezing temperature (Vali, 2014; Wright and Petters, 2013). Thus, the droplets containing high Snomax concentrations (dashed portion of the $\bar{g}_1$ curve shown in Fig. 3). The tail of the broader $\bar{g}_1$ distribution spreads into a lower (more active) contact angle range than the much narrower $\bar{g}_2$ distribution does. Furthermore, the broader $\bar{g}_1$ distribution of HIN activity can be interpreted as being consistent with the finding by Polen et al. (2016) that the more active Type I protein aggregates (which are described by the first distribution, $\bar{g}_1$) are less stable over storage time and refreezes than the Type III protein aggregates (which are described by the second distribution, $\bar{g}_2$). A broader distribution implies that freezing is determined by a wider (more variable) range of HIN activity and thus a wider diversity of the INP's properties. The freezing induced by the more active $\bar{g}_1$ distribution is understood to be caused by rare and very active large proteins aggregates that would arguably be more susceptible to deterioration, weakening, and decay over time. This would produce a wider diversity in HIN activity, which would result in the broader $\bar{g}_1$ normal distribution of contact angles (Fig. 3) that we derived from analysis of the droplet freezing spectra in Fig. 2.

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3.2 Mixtures of Snomax and Illite particles

The second part of this study investigates the applicability of the mixing model to a mixture of Snomax bacterial and illite mineral particles, recalling that Snomax is itself a mixture of at least two distinct types of INPs. Figure 5 shows two droplet freezing spectra retrieved from droplets generated from a mixture of Snomax and illite: a 1:1 mixture of 5x10^{-7} wt% Snomax and 0.1 wt% illite, and a 1:1 mixture of 1x10^{-7} wt% Snomax and 0.1 wt% illite. The two frozen fraction curves are plotted along with the other Snomax frozen fraction curves and another spectrum retrieved from a 0.05 wt% illite suspension. As the Snomax-illite mixtures were prepared by mixing equal amounts of each component, the effective concentration of each is half its original concentration. That is why a 0.05 wt% illite frozen fraction curve is included in Fig. 4 as it should better resemble the illite concentration in the mixture. It was shown in Beydoun et al. (2016) that for

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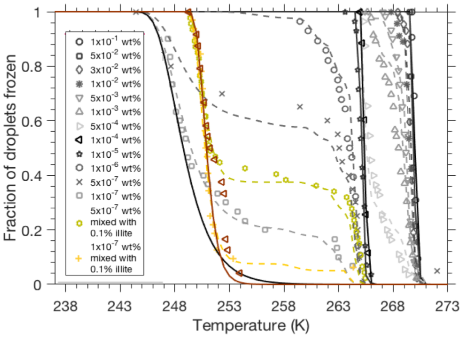

Figure 5. All droplet freezing spectra for Snomax-containing droplets from Fig. 1 shown in grey, along with the frozen fraction curves corresponding to 0.05 wt% illite (dark red), a 1:1 mixture of 5x10^{-7} wt% Snomax and 0.1 wt% illite (yellow), and a 1:1 mixture of 1x10^{-7} wt% Snomax and 0.1 wt% illite (orange). The modelled frozen fraction curve for the 0.05 wt% illite frozen fraction curve is also plotted (solid dark-red line) along with the fits for the frozen fraction curves corresponding to the illite-Snomax mixtures (yellow and orange dashed lines). Solid black lines on the right are frozen fraction curves that can be described by a $\bar{g}$ distribution ($\bar{g}_1$ and $\bar{g}_2$). The solid black line on the left is the modelled frozen fraction curve of the background impurities.

this method and a 0.03 wt% illite concentration, the resultant freezing curve can be modeled using illite's previously retrieved $\bar{g}$ ($\mu_{illite} = 1.72, \sigma_{illite} = 0.122$) and surface area of A_{illite} = 2.0 x 10^{-2} cm^2. Illite is above its critical area threshold at this concentration. This predicted curve using illite's $\bar{g}$ distribution is also shown in Fig. 5. At this concentration, illite does not induce freezing until < 254 K, well below the critical temperature range of Snomax's weaker $\bar{g}_2$ distribution that is observed > 260 K.

The mixing model expressed by Eqn. (6) is applied to the droplet frozen fraction curves corresponding to the mixtures of Snomax and illite. In this case the contribution from the background impurities is ignored, which is thought to be a good assumption as the tail of the frozen fraction curves aligns quite well with the frozen fraction curve for droplets containing only 0.05 wt% illite. Random sampling takes place from $\bar{g}_2$ for Snomax to produce the $g*$ distributions needed for the first term inside the exponential in Eqn. (6) since these Snomax concentrations are below the $\bar{g}_2$ distribution's critical area threshold, while the second term uses $\bar{g}_{illite}$ and A_{illite} that were previously determined as discussed above for illite above its critical area. The consequent n_{draws} values that result in the closest modelled frozen fraction curves for each of the experimental frozen fraction curves produced from a Snomax-illite mixture are: 1190 for 5x10^{-7} wt% Snomax mixed with 0.1 wt% illite, and 500 for 1x10^{-7} wt% Snomax mixed with 0.1 wt% illite. The model produces frozen fraction curves that encompass the freezing behavior observed for the Snomax-illite mixtures quite well, with an initial increase in the fraction of droplets frozen due to the contribution from the HIN activity of Snomax in the same way the frozen fraction of droplets containing only Snomax behaved. This is followed by an approximate plateau in the frozen fraction curve indicating the absence of any Snomax INP. It should be noted that the reduction in the intermediary plateau of the fraction of droplets frozen containing the Snomax-lllte mixture compared to the droplets containing only Snomax can be explained by the reduction of effective Snomax concentration in the mixture due to diluting the concentrations of the 0.1 wt% illite and the 5x10^{-7} wt% Snomax as well as the 1x10^{-7} wt% Snomax by half upon making 1:1 solutions of each. While Augustin et al. (2016) speculated that the reduction in freezing temperatures upon mixing birch pollen wash water with an illite solution may have had to do with the resultant mixing state of the generated particles, no evidence of this is found in our measurements. The mixing model presented here is therefore only the second HIN

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model that accounts for multiple independent distributions of HIN, and the first to do so for a system comprised of four distinct distributions of HIN activity (the two distributions of HIN activity of Snomax, that of illite, and that of the background impurities) spanning seven orders of magnitude of particle material concentration and a temperature range of 271 K down to 248 K.

Due to Snomax's well-defined cutoff in HIN activity at low concentrations where no freezing is induced below 258 K, there is really no temperature regime in which the INP's contained in illite and Snomax compete. In other words, it is not possible to dilute Snomax to a point where its HIN activity is expressed in the temperature range where illite's HIN activity becomes significant. The measurements do clearly indicate that there is a high temperature cutoff for Snomax's contribution to freezing and the mixing model does a good job of producing this behavior.

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A droplet-particle system examined here that deserves greater attention is the 1:1 mixture of $1x10^{-7}$ wt% Snomax and 0.1 wt% illite. At this effective Snomax concentration of $5x10^{-8}$ wt%, the volume equivalent diameter of total surface area of Snomax particles present is around 550 nm (for an assumed 1 g/cm^3 density). Hartmann et al. (2013) investigated the HIN activity of size selected Snomax particles using the LACIS cloud simulator (Stratmann et al., 2004). Their results were somewhat consistent with what was found here as their 650 nm and 800 nm size-selected particles produced a frozen fraction curve that plateaued at 10% of droplets frozen below 263 K. Smaller Snomax particle sizes they investigated (100 nm, 300 nm, 400 nm) caused freezing at lower temperatures between 263 K and 258 K and were generally found to be rarer than the larger particles in the polydisperse aerosol size distribution. This may explain why upon decreasing the Snomax concentration in the experiments presented here, the droplets retained freezing at the higher temperatures of 265 K to 263 K and not the lower temperatures of 263 K to 258 K as shown in frozen fraction curves corresponding to droplets containing $1x10^{-7}$ wt% Snomax, and $1x10^{-7}$ wt% Snomax mixed with 0.1 wt% illite. Therefore, in the context of atmospheric aerosol, at the limit of a very low probability of finding a

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biological particle, the INP’s represented by the less active but more abundant Snomax $\bar{g}_2$ distribution and their colder critical temperatures are the most likely to be found (in the limiting assumption of Snomax being a reasonable proxy of biological INPs). This is what the lower Snomax concentration frozen fraction curve associated with the Snomax-illite mixture resembles; even when Snomax particles become scarce they still induce a high freezing temperature in the small fraction of droplets that still contain them (around 5% in the frozen fraction curve being examined).

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The question then of what ratios of Snomax and illite are needed for one to control the HIN activity over the other becomes irrelevant. The existence of any Snomax particles in a droplet will shift the resultant freezing to a much higher temperature regime and no amount of mineral dust can practically compete with that. This understanding provides more quantitative validation of the conventional wisdom that a relatively rare biological particle exhibits exceptional freezing behavior, overwhelming other non-biological components. Augustin et al. (2016) provided evidence of this but it remained unclear whether the illite in the illite/birch pollen wash water mixture investigated was contributing to freezing at all, as the resultant frozen fraction curve of the mixture merely looked like a weaker version of the frozen fraction curve retrieved from the droplets comprised purely of birch pollen wash water (identical shape but with a lower freezing temperatures). This arguably limits the ability to validate a mixing model to a system comprised of a mixture of INPs. It could also be argued that probing only one size and thus surface area of INPs in their study is another limitation and varying these parameters could have helped discern whether differences in the resultant frozen fraction curves between the mixtures and the pure components were due to differences in the amount of INP material present or due to potential mixing state alteration.

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From the perspective of modelling multi-component heterogeneous ice nucleating particles, it remains a subject of further research to determine how many $\bar{g}$ distributions are necessary to encompass the freezing behavior of mixed particle systems at low temperatures where dust is known to be quite ice active. However, the work presented here supports the notion that two distributions of HIN activity are necessary to distinguish between the dust and biological regimes when illite and Snomax are used as proxies for these two types of INP. Regarding the motivating hypothesis that small biological particles can enhance the freezing capabilities of mineral dust surfaces, it can be concluded that the effectiveness of Snomax enhancing illite or any mineral dust surface is limited by its ability to partition itself and thus allow its HIN activity to be manifested externally. Hypothetical scenario 3, that was introduced in Section 1.2 and depicted in Fig. 1b, in which the limited amount of biological material does not distribute itself among all droplets, therefore closely resembles the bio-dust mixture proxy examined here. This is in contrast to the more effective behavior of soil borne fungus investigated in O'Sullivan et al. (2016). The biological macromolecules in that study were shown to bind onto clay and thus partition themselves among the dust surfaces, thereby distributing their HIN activity externally. This difference between Snomax and soil borne fungus should be kept in mind when considering the relevance of dust-bio mixtures in the atmosphere, and future work can attempt to quantify whether the ability of soil born fungus to adsorb unto clay surfaces can render it a stronger INP than Snomax despite its weaker HIN activity.

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4. Conclusions

A new heterogeneous ice nucleation (HIN) mixing model was formulated to better understand and predict how cloud droplet systems containing more than one component of INPs behave. The new model successfully predicted the freezing spectra of droplets containing Snomax bacterial particles as well as a mixture of Snomax and illite NX mineral particles, a proxy for bio-dust particle mixtures in the atmosphere. Snomax was characterized by two distributions of HIN activity, a model consistent with Snomax's proposed ice nucleating Type I and Type III protein aggregates. The first distribution exhibited a broader contact angle range and therefore had a more gradual decay in its contribution to droplet freezing with decreasing concentration compared to the second distribution that exhibited a very narrow range of contact angles. The

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broadness of the first distribution is likely related to the INPs' instability as the very rare and most active INPs can noticeably deteriorate over time as recently reported by Polen et al. (2016).

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Upon examining droplets containing a mixture of illite mineral particles and a low concentration of Snomax, the resultant frozen fractions merely followed freezing as dictated by the HIN activity of each individual component; there was no evidence of an intermediary freezing temperature range produced by mixing the two types of INPs. This result is similar to the one reported in Augustin-Bauditz et al. (2016) using a mixture of birch pollen wash water and illite. The absence of a broad range of nucleating temperatures connecting the two HIN regimes is partly due to the sharpness of the second Snomax distribution of HIN activity. The inability of Snomax bacterial particles to further distribute themselves externally among all droplets is what led the mixture to exhibit the two distinct regimes of freezing due to Snomax and illite respectively. Therefore, in the limiting assumption that the system examined here is a close proxy to real atmospheric bio-dust mixtures, it would be safe to assume that the limiter to high temperature heterogeneous freezing in the atmosphere is merely the existence of biological material in the droplet. More likely than not no compromise would be made in the quality of biological material's HIN activity when freezing is induced due to the presence of other particle components, such as the illite minerals studied here.

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Acknowledgements. This research was supported by the National Science Foundation (CHE-1554941). Hassan Beydoun was partially supported by the Bertucci Fellowship from the College of Engineering at Carnegie Mellon University. Michael Polen was supported by a Graduate Research Fellowship from the National Science Foundation.

References

Ariya, P. a., Sun, J., Eltouny, N. a., Hudson, E. D., Hayes, C. T. and Kos, G.: Physical and chemical characterization of bioaerosols – Implications for nucleation processes, Int. Rev. Phys. Chem., 28(1), 1–32, doi:10.1080/01442350802597438, 2009.

Atkinson, J. D., Murray, B. J., Woodhouse, M. T., Whale, T. F., Baustian, K. J., Carslaw, K. S., Dobbie, S., O'Sullivan, D. and Malkin, T. L.: The importance of feldspar for ice nucleation by mineral dust in mixed-phase clouds, Nature, 498(7454), 355–358, doi:10.1038/nature12278, 2013.

Augustin-Bauditz, S., Wex, H., Denjean, C., Hartmann, S., Schneider, J., Schmidt, S., Ebert, M. and Stratmann, F.: Laboratory-generated mixtures of mineral dust particles with biological substances: characterization of the particle mixing state and immersion freezing behavior, Atmos. Chem. Phys., 16(9), 5531–5543, doi:10.5194/acp-16-5531-2016, 2016.

Bühl, J., Ansmann, A., Seifert, P., Baars, H. and Engelmann, R.: Toward a quantitative characterization of heterogeneous ice formation with lidar/radar: Comparison of CALIPSO/CloudSat with ground-based observations, Geophys. Res. Lett., 40(16), 4404–4408, doi:10.1002/grl.50792, 2013.

Cantrell, W. and Heymsfield, A.: Production of Ice in Tropospheric Clouds: A Review, Bull. Am. Meteorol. Soc., 86(6), 795–807, doi:10.1175/BAMS-86-6-795, 2005.

Christner, B. C., Morris, C. E., Foreman, C. M., Cai, R. and Sands, D. C.: Ubiquity of Biological Ice Nucleators in Snowfall, Science (80-.)., 319(5867), 1214–1214, doi:10.1126/science.1149757, 2008.

Creamean, J. M., Suski, K. J., Rosenfeld, D., Cazorla, A., Demott, P. J., Sullivan, R. C., White, A. B., Ralph, F., Minnis, P., Comstock, J. M., Tomlinson, J. M. and Prather, K. A.: Dust and biological aerosols from the Sahara and Asia influence precipitation in the Western U.S., Science (80-.)., doi:10.1126/science.1227279, 2013.

DeMott, P. J. and Prenni, A. J.: New Directions: Need for defining the numbers and sources of biological aerosols acting as ice nuclei, Atmos. Environ., 44(15), 1944–1945, doi:10.1016/j.atmosenv.2010.02.032, 2010.

DeMott, P. J., Prenni, A. J., McMeeking, G. R., Sullivan, R. C., Petters, M. D., Tobo, Y., Niemand, M., Möhler, O., Snider, J. R., Wang, Z. and Kreidenweis, S. M.: Integrating laboratory and field data to quantify the immersion freezing ice nucleation activity of mineral dust particles, Atmos. Chem. Phys., 15(1), 393–409, doi:10.5194/acp-15-393-2015, 2015.

Ervens, B. and Feingold, G.: On the representation of immersion and condensation freezing in cloud models using different nucleation schemes, Atmos. Chem. Phys., 12, 5807–5826, doi:10.5194/acp-12-5807-2012, 2012.

Franc, G. D. and Demott, P. J.: Cloud Activation Characteristics of Airborne Erwinia carotovora Cells, J. Appl. Meteorol., 37(10), 1293–1300 doi: 10.1175/1520-0450(1998)037%3C1293:CACOAE%3E2.0.CO;2, 1998.

Fröhlich-Nowoisky, J., Hill, T. C. J., Pummer, B. G., Yordanova, P., Franc, G. D. and Pöschl, U.: Ice nucleation activity in the widespread soil fungus Mortierella alpina, Biogeosciences, 12(4), 1057–1071, doi:10.5194/bg-12-1057-2015, 2015.

Hartmann, S., Augustin, S., Clauss, T., Wex, H., Šantl-Temkiv, T., Voigtländer, J., Niedermeier, D. and Stratmann, F.: Immersion freezing of ice nucleation active protein complexes, Atmos. Chem. Phys., 13(11), 5751–5766, doi:10.5194/acp-13-5751-2013, 2013.

Hiranuma, N., Augustin-Bauditz, S., Bingemer, H., Budke, C., Curtius, J., Danielczok, A., Diehl, K., Dreischmeier, K., Ebert, M., Frank, F., Hoffmann, N., Kandler, K., Kiselev, A., Koop, T., Leisner, T., Möhler, O., Nillius, B., Peckhaus, A., Rose, D., Weinbruch, S., Wex, H., Boose, Y., DeMott, P. J., Hader, J. D., Hill, T. C. J., Kanji, Z. A., Kulkarni, G., Levin, E. J. T., McCluskey, C. S., Murakami, M., Murray, B. J., Niedermeier, D., Petters, M. D., O'Sullivan, D., Saito, A., Schill, G. P., Tajiri, T., Tolbert, M. A., Welti, A., Whale, T. F., Wright, T. P. and Yamashita, K.: A comprehensive laboratory study on the immersion freezing behavior of illite NX particles: a comparison of 17 ice nucleation measurement techniques, Atmos. Chem. Phys., 15(5), 2489–2518, doi:10.5194/acp-15-2489-2015, 2015a.

Hiranuma, N., Möhler, O., Yamashita, K., Tajiri, T., Saito, A., Kiselev, A., Hoffmann, N., Hoose, C., Jantsch, E., Koop, T. and Murakami, M.: Ice nucleation by cellulose and its potential contribution to ice formation in clouds, Nat. Geosci., 8(4), 273–277, doi:10.1038/ngeo2374, 2015b.

Hoose, C., Lohmann, U., Erdin, R. and Tegen, I.: The global influence of dust mineralogical composition on heterogeneous ice nucleation in mixed-phase clouds, Environ. Res. Lett., 3(2), 25003, doi:10.1088/1748-9326/3/2/025003, 2008.

Kamphus, M., Ettner-Mahl, M., Klimach, T., Drewnick, F., Keller, L., Cziczo, D. J., Mertes, S., Borrmann, S. and Curtius, J.: Chemical composition of ambient aerosol, ice residues and cloud droplet residues in mixed-phase clouds: single particle analysis during the Cloud and Aerosol Characterization Experiment (CLACE 6), Atmos. Chem. Phys., 10(16), 8077–8095, doi:10.5194/acp-10-8077-2010, 2010.

Möhler, O., DeMott, P. J., Vali, G. and Levin, Z.: Microbiology and atmospheric processes: the role of biological particles in cloud physics, Biogeosciences Discuss., 4(4), 2559–2591, doi:hal-00297909, 2007.

Morris, C. E., Georgakopoulos, D. G. and Sands, D. C.: Ice nucleation active bacteria and their potential role in precipitation, J. Phys. IV, 121, 87–103, doi:10.1051/jp4:2004121004, 2004.
Murray, B. J., O'Sullivan, D., Atkinson, J. D. and Webb, M. E.: Ice nucleation by particles immersed in supercooled cloud droplets, Chem. Soc. Rev., 41(19), 6519, doi:10.1039/c2cs35200a, 2012.
Niedermeier, D., Shaw, R. a., Hartmann, S., Wex, H., Clauss, T., Voigtländer, J. and Stratmann, F.: Heterogeneous ice nucleation: exploring the transition from stochastic to singular freezing behavior, Atmos. Chem. Phys., 11(16), 8767–8775, doi:10.5194/acp-11-8767-2011, 2011.

O′Sullivan, D., Murray, B. J., Ross, J. F., Whale, T. F., Price, H. C., Atkinson, J. D., Umo, N. S. and Webb, M. E.: The relevance of nanoscale biological fragments for ice nucleation in clouds, Sci. Rep., 5, 8082, doi:10.1038/srep08082, 2015.

O'Sullivan, D., Murray, B. J., Ross, J. F. and Webb, M. E.: The adsorption of fungal ice-nucleating proteins on mineral dusts : a terrestrial reservoir of atmospheric ice-nucleating particles, Atmos. Chem. Phys., 16, 7879–7887, doi:10.5194/acp-16-7879-2016, 2016.

Petters, M. D. and Wright, T. P.: Revisiting ice nucleation from precipitation samples, Geophys. Res. Lett., 42(20), 8758–8766, doi:10.1002/2015GL065733, 2015.

Polen, M., Lawlis, E. and Sullivan, R. C.: The unstable ice nucleation properties of Snomax(R) bacterial particles, J. Geophys. Res. - Atmos., 121, 11,666–11,678, doi:10.1002/ 2016JD025251, 2016.

Prather, K. a, Bertram, T. H., Grassian, V. H., Deane, G. B., Stokes, M. D., Demott, P. J., Aluwihare, L. I., Palenik, B. P., Azam, F., Seinfeld, J. H., Moffet, R. C., Molina, M. J., Cappa, C. D., Geiger, F. M., Roberts, G. C., Russell, L. M., Ault, A. P., Baltrusaitis, J., Collins, D. B., Corrigan, C. E., Cuadra-Rodriguez, L. a, Ebben, C. J., Forestieri, S. D., Guasco, T. L., Hersey, S. P., Kim, M. J., Lambert, W. F., Modini, R. L., Mui, W., Pedler, B. E., Ruppel, M. J., Ryder, O. S., Schoepp, N. G., Sullivan, R. C. and Zhao, D.: Bringing the ocean into the laboratory to probe the chemical complexity of sea spray aerosol., Proc. Natl. Acad. Sci. U. S. A., 110(19), 7550–5, doi:10.1073/pnas.1300262110, 2013.

Pratt, K. a., DeMott, P. J., French, J. R., Wang, Z., Westphal, D. L., Heymsfield, A. J., Twohy, C. H., Prenni, A. J. and Prather, K. a.: In situ detection of biological particles in cloud ice-crystals, Nat. Geosci., 2(6), 398–401, doi: 10.1038/ngeo521, 2009.

Schnell, R. C. and Vali, G.: Biogenic Ice Nuclei: Part I. Terrestrial and Marine Sources, J. Atmos. Sci., 33(8), 1554–1564, doi:10.1175/1520-0469(1976)033<1554:BINPIT>2.0.CO;2, 1976.

Turner, M. A., Arellano, F. and Kozloff, L. M.: Three separate classes of bacterial ice nucleation structures, J. Bacteriol., 172(5), 2521–2526, doi:PMC208892, 1990.

Vali, G.: Freezing Nucleus Content of Hail and Rain in Alberta, J. Appl. Meteorol., 10(1), 73–78, doi:10.1175/1520-0450(1971)010<0073:FNCOHA>2.0.CO;2, 1971.

Vali, G.: Ice nucleation—A review. Nucleation and Atmospheric Aerosols, M. Kulmala and P. Wagner, Eds., Pergamon, 271–279, 1996.

Vali, G.: Interpretation of freezing nucleation experiments: Singular and stochastic; Sites and surfaces, Atmos. Chem. Phys., 14(11), 5271–5294, 2014.

Welti, A., Lüönd, F., Kanji, Z. A., Stetzer, O. and Lohmann, U.: Time dependence of immersion freezing: an experimental study on size selected kaolinite particles, Atmos. Chem. Phys., 12(20), 9893–9907, doi:10.5194/acp-12-9893-2012, 2012.

Wex, H., Augustin-Bauditz, S., Boose, Y., Budke, C., Curtius, J., Diehl, K., Dreyer, a., Frank, F., Hartmann, S., Hiranuma, N., Jantsch, E., Kanji, Z. a., Kiselev, a., Koop, T., Möhler, O., Niedermeier, D., Nillius, B., Rösch, M., Rose, D., Schmidt, C., Steinke, I. and Stratmann, F.: Intercomparing different devices for the investigation of ice nucleating particles using Snomax® as test substance, Atmos. Chem. Phys., 15(3), 1463–1485, doi:10.5194/acp-15-1463-2015, 2015.

Wolber, P. K., Deininger, C. A., Southworth, M. W., Vandekerckhove, J., van Montagu, M. and Warren, G. J.: Identification and purification of a bacterial ice-nucleation protein., Proc. Natl. Acad. Sci., 83(19), 7256–7260, doi:10.1073/pnas.83.19.7256, 1986.

Wright, T. P. and Petters, M. D.: The role of time in heterogeneous freezing nucleation, J. Geophys. Res.

Atmos., 118(9), 3731–3743, doi: 10.1002/jgrd.50365, 2013.

Yankofsky, S. a., Levin, Z., Bertold, T. and Sandlerman, N.: Some Basic Characteristics of Bacterial Freezing Nuclei, J. Appl. Meteorol., 20(9), 1013–1019, doi:10.1175/1520-0450(1981)020<1013:SBCOBF>2.0.CO;2, 1981.

Zobrist, B., Koop, T., Luo, B. P., Marcolli, C. and Peter, T.: Heterogeneous ice nucleation rate coefficient of water droplets coated by a nonadecanol monolayer, J. Phys. Chem. C, 111(1), 2149–2155, doi:10.1021/jp066080w, 2007.

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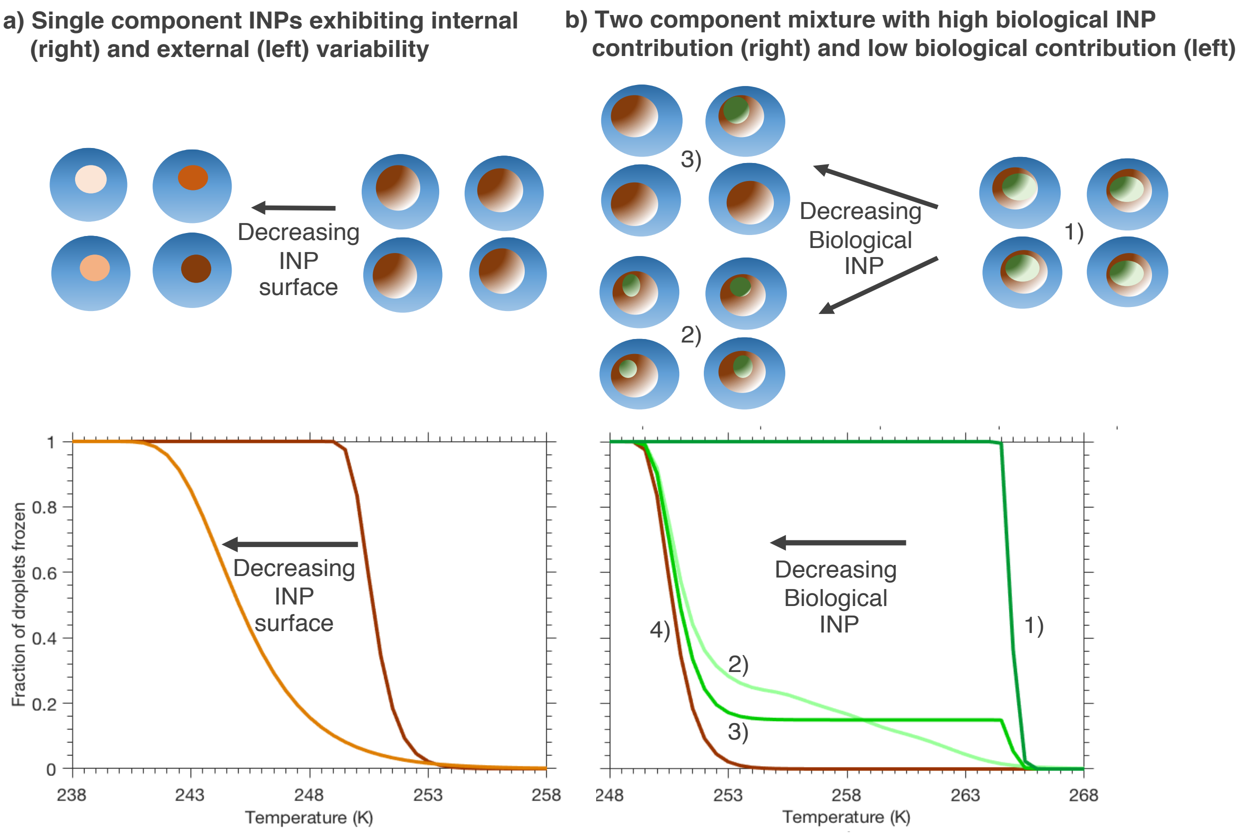
a) Single component INPs exhibiting internal (right) and external (left) variability

b) Two component mixture with high biological INP contribution (right) and low biological contribution (left)

Figure 1. a) Top: On the right-hand side droplets containing particle surfaces larger than the component's critical surface area are illustrated with the color gradient depicting internal variability in HIN activity. On the left-hand side droplets now contain particle surfaces smaller than the species' critical surface area following dilution and the particles exhibit external variability in HIN depicted by the different colors. **a) Bottom:** The droplet temperature freezing spectrum predicted using the $\bar{g}$ framework. Decreasing surface area leads to higher external variability in HIN activity and thus a wider range over which droplets freeze. **b) Top:** On the right-hand side droplets contain a mixture of mineral dust (brown) and biological (green) particle surfaces, both of which are larger than their component's critical surface area. On the left-hand side two hypothetical scenarios of reduced biological particle surface areas are depicted as the concentration of biological INP is reduced. In scenario (2) the biological material distributes itself equally among the droplets and thus remains internally mixed while in scenario (3) the biological material does not further partition itself further and becomes externally mixed. Scenario (4) represents the absence of any biological material and the resultant temperature freezing spectrum is identical to the one for dust lying above its critical surface area shown in the bottom of Fig. 1a. **b) Bottom:** The predicted impact of the INP concentration and mixing state of the dust-bio mixture on the resultant freezing spectrum of droplets containing the particle mixture.